Forty Years of Achievement in Heart, Lung, and Blood Research

National Heart, Lung, and
Blood Institute

National Institutes of Health
September 1987

This publication was made possible through an educational grant from Parke-Davis, a Division of Warner-Lambert Company.

"Whereas the Congress hereby finds and declares that the Nation's health is seriously threatened by diseases of the heart and circulation including high blood pressure, which annually kill over five hundred and eighty-eight thousand of our people and disable approximately seven million eight hundred thousand more. These diseases are the main cause of death in the United States and more than one in every three of our people die from them; and

"Whereas it is therefore the policy of the United States to provide for research and control relating to diseases of the heart and circulation in a supreme endeavor to develop speedily more effective means of prevention, diagnosis, and treatment of such diseases: Now, therefore

"Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled, that this Act may be cited as the 'National Heart Act.'"

U.S. Senate
S. 2215
1948

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Forty Years of Achievement in Heart, Lung, and Blood Research

A collection of essays in selected areas of biomedical research accomplishment

Prepared by the
National Heart, Lung,
and Blood Institute
in celebration of its
Fortieth Anniversary
and the Centennial of the
National Institutes of Health

September 1987

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Foreword

In honor of the fortieth anniversary of the National Heart, Lung, and Blood Institute and the centennial celebration of the National Institutes of Health, I am pleased to present this commemorative volume and its companion, entitled "A Salute to the Past."

These publications speak not only to the Institute's achievements but also to how its organization evolved over the years and how its goals have changed in response to emerging national health needs, new technologies, and increasingly sophisticated approaches to biomedical research. In reaching out to the community and servicing the Nation, the Institute's accomplishments reflect the wisdom and dedication of its intramural and extramural staff, cooperation from a concerned research community, understanding and supportive legislative and executive leadership, and cooperation, and often tangible support, from health-related commercial organizations.

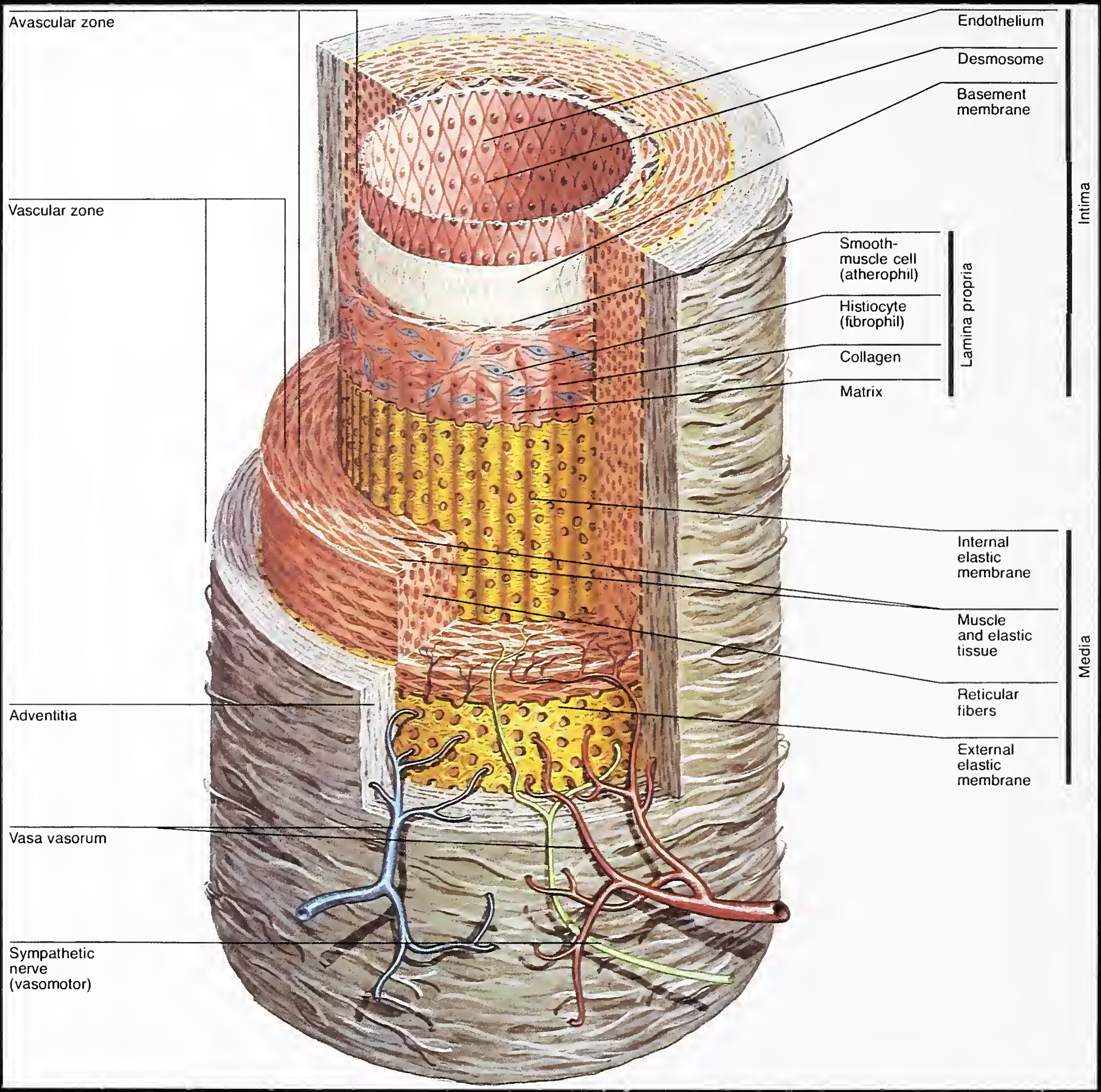
One of the most noteworthy aspects of the Institute's early years was its broad approach to health problems. Both commemorative volumes bear testimony to important features of the Institute from its earliest years when its budget was small but its vision expansive and its approach eclectic. From the beginning, the intramural as well as the extramural programs emphasized basic research. Indeed, this basic research, in several instances, was significant enough to earn Nobel prizes. Because of the importance of primate models, the Institute was among the first sources of support for primate colonies. It was one of the first to contribute to development of what has become the Medlars facility, and was among the first of NIH Institutes to recognize the importance and relevance of behavioral approaches to biomedical research. In short, the Institute's objective always has been to improve our Nation's health. It has achieved this through open-minded, innovative planning and a commitment to a balanced program.

The title of this volume, 40 Years of Achievement in Heart, Lung, and Blood Research, *promises more than possibly can be presented in a commemorative publication. The achievements in heart, lung, and blood research have been amply documented in the scientific literature over the years. This volume describes the authors' personal reflections of achievements in eight research areas. The authors were asked to develop their topics from their personal experiences, knowledge, and commitment over the years. The areas selected are representative of the many significant advances in research in which the Institute has been involved. The articles are addressed to a variety of audiences—basic and clinical scientists, health professionals, and the public.*

The progress presented here illustrates a process and a philosophy that have prevailed since the Institute's inception. Although the ultimate goal has always been betterment of national health, the path for realization was often through innovative basic research, even when not directly related to heart, lung, or blood diseases. Other advances were achieved through population-based studies, through behavioral research, frequently through clinical trials and, most recently, through outreach programs to inform practicing physicians and the public.

In presenting these volumes, it is with thanks to all those who have made possible the Institute's achievements over the years. Ideas are essential, but without the ability, interest, and dedication of people—they will come to naught. And so, on behalf of the Institute, I thank all those who have made these advances possible.

Claude Lenfant, M.D.
Director
National Heart, Lung, and Blood Institute


Avascular zone
Vascular zone
Adventitia
Vasa vasorum
Sympathetic nerve (vasomotor)
Endothelium
Desmosome
Basement membrane
Smooth-muscle cell (atherophil)
Histiocyte (fibrophil)
Collagen
Matrix
Lamina propria
Intima
Internal elastic membrane
Muscle and elastic tissue
Reticular fibers
External elastic membrane
Media

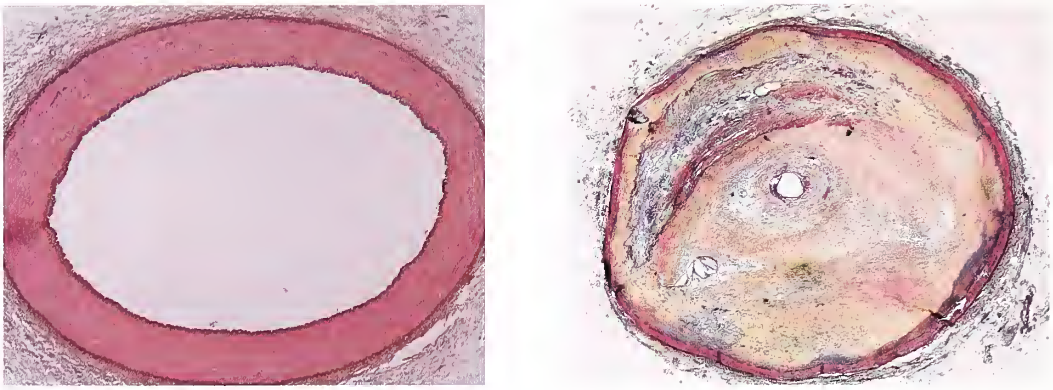
Atherosclerosis Research: Decades of Progress

Schematic structural characteristics of the normal arterial wall.

The years of the Second World War were a period of relative inactivity in research on atherosclerosis. A notable exception were the studies of Yates and his associates describing autopsy findings on coronary artery disease in men 18 to 39 years of age who had died in the military service. The field became active again in the late 1940s, and progress was rapid. Our knowledge at that time was summarized in a review published in 1935, which presented evidence to refine the current infiltration theory of atherosclerosis. The theory held that most of the lipid in atherosclerotic plaques was not formed in the artery but was brought there from elsewhere, namely, from the lipids carried in the blood infiltrating the vessel wall. The review stated:

"The initial stage in the development of human arteriosclerosis consists of local changes in the walls of the arteries themselves, changes which are responsible for the subsequent precipitation of lipids in the affected areas. In man, as in the rabbit, there is every reason to believe that these changes follow as the result of some form of injury to the arterial walls. As to the cause of this injury, the experimental data yield hardly any information which is capable of application in the human being. . . . Following the initial local injury, lipids are deposited in the injured intima especially in the intercellular substances. These deposits stir into activity numerous macrophages which are attracted to the site and which then engulf a large part of the lipoid material. It seems entirely possible that the free lipoid deposits can stimulate the proliferation of connective tissue cells in the intima. This effect is added to that of the original injury to produce a reparative fibrous tissue reaction in the affected areas. If the original injury that initiated the whole process ceases to operate, it is probable that the lipid deposits prevent immediate healing and continue to exert their influence so that the lesions persist progressing slowly. Under these conditions, however, it seems likely from the experimental evidence that the lipids may be slowly removed and may even disappear finally, leaving only a fibrous thickening of the intima.

Hypercholesterolemia is not found with any regularity in association with arteriosclerosis in man, . . . but there seems to be a general impression that a disturbance



Normal and atherosclerotic vessels.

in lipoid metabolism must be postulated to explain the accumulation of lipids in the lesions of arteriosclerosis. This impression may prove to be correct, but there is no concrete evidence at present to show that it is . . . . If a disturbance in lipoid metabolism participates in the etiology of arteriosclerosis in man, the fact remains to be demonstrated in the future.”

Research to understand the changes of arteriosclerosis in the vessel wall was slow to progress. Aside from a few early observations, only in the past 15 years has there been significant and rapid progress in understanding some of the many biological mechanisms that go into building arteriosclerotic plaques. The question of the association of lipids with the development of atherosclerosis in humans was brought under scrutiny, and the uncertainty and lack of concrete data noted above soon were resolved. Research on lipids proved so rewarding that the field expanded greatly and continues to be very productive.

Associations Between Lipids and Atherosclerotic Disease

The Framingham Heart Study was planned in 1947. It examined its first subject in 1948 and came under the new National Heart Institute (NHI) in July 1949. Framingham had been selected, in part, because it had been the site of a community study of tuberculosis from 1917 to 1923. The study focused on arteriosclerotic and hypertensive cardiovascular diseases. It was assumed that these were the result of multiple causes that work slowly within the individual. About 5,000 persons free of overt disease have been observed carefully and repeatedly for many years. They received customary medical care in their normal community until sizable numbers acquired cardiovascular disease. The prospectively collected data were searched for those antecedent factors that could be identified as influencing this development. The concept of risk factors was thus born and, over the years, incontrovertible data were presented and quantitated showing that *age, male sex, blood cholesterol level, high blood pressure, cigarette smoking,* and *diabetes* were characteristics that could be expressed as numerical probabilities for having heart attack or strokes in the future.

It takes time in such prospective community cohort studies to accumulate sufficient data to analyze, and Framingham was no exception. However, a collation and systematic account of major findings was begun with the report of the first 14 years of follow-up. The first report of a positive association of blood cholesterol level and heart attack was made in 1956 by the Technical Group of the Committee on Lipoproteins and Atherosclerosis of the National Advisory Heart Council, NHI. Subsequently, reports from Framingham, Los Angeles, and a study of civil servants in Albany, New York, were published together in 1957.

The Framingham study has had and continues to have immense, worldwide influence in both public health concepts and patient care. Even though it does not stand alone (between 1948 and 1960, 7 large prospective studies were begun, totaling about 11,000 persons aged 40-59 years), the Framingham Heart Study has been a pioneer and premier study that speaks to the associations of certain characteristics of humans, such as blood cholesterol level and blood pressure and the subsequent risk or probability of disease. It has been one of the bases for the design of clinical trials of hypertensive agents, of hypocholesterolemic drugs or diet, and of the current National High Blood Pressure Education Program and National Cholesterol Education Program.

Diet, Blood Cholesterol, and Cardiovascular Disease

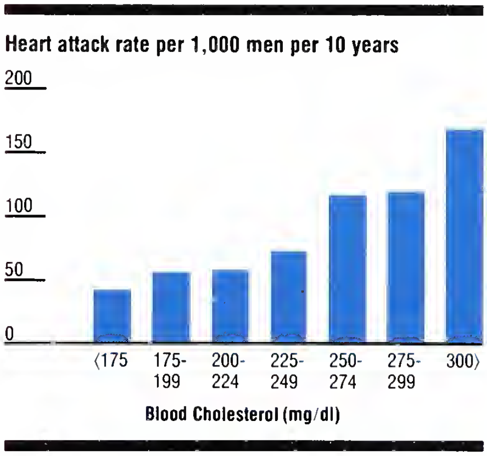
Early attempts to alter blood cholesterol levels by increasing dietary cholesterol were not informative. For example, one study reported in 1950 failed to find consistent effects from the month-long feeding of 30 grams of cholesterol per day to elderly men. While it is known today that dietary cholesterol does have a positive association with blood cholesterol, such early studies turned attention to the stronger associations with dietary total fat and saturated fat and the relative hypocholesterolemic effects of polyunsaturated fats.

The cholesterol-lowering effect of vegetarian diets and liquid vegetable oils in humans was reported in 1952 and subsequent years. By the late 1950s, results of many controlled metabolic dietary experiments in humans led scientists to state, in the form of simple equations or formulae, the effects of various dietary fat combinations on blood cholesterol level. These equations, which have been shown to predict rather closely the average effect of qualitative and quantitative changes in the fat composition of the diet, have become important tools for public health and medical practice applications as well as for research purposes. Thus, it became established that diets high in saturated fat promoted hypercholesterolemia, low-fat diets reduced it, saturated fats tended to increase blood cholesterol, polyunsaturated fats had the opposite effect, and oleic acid (a monoene found in large quantity in olive oil) appeared neutral toward the blood cholesterol level. Moreover, an increase in dietary cholesterol has the effect of increasing blood cholesterol.

Expanding the Information Base

Clinical research on lipid metabolism was paralleled by cohort studies of an epidemiologic nature. Some utilized populations with special traditional dietary behavior or lifestyle. Vegetarians, for example, have lower blood lipid levels than nonvegetarians, and strict vegetarians lower levels still. Other studies were undertaken in monasteries and with Seventh Day Adventists.

The most informative studies have been international prospective cohort studies. Of these, the Seven Countries study has offered multiple opportunities to derive, compare, and contrast risk factor associations. It has been of particular value in displaying the influence of dietary fats. The study took its genesis from a prospective study started in 1947 among business and professional men in Minnesota. The variables that might be predictive of cardiovascular disease were extended to several countries and field tested and, by 1957, a full-dress study of methods and organization was made.



Risk of heart attack increases as blood cholesterol increases.

A prospective study of nearly 13,000 men in 16 groups and in 6 Western countries and Japan was initiated between 1958 and 1964. In general, the research confirmed the risk factor associations identified by other studies, but it also provided extensive data that dietary fat intake both by quantity and type was a determinant of blood cholesterol level in free-living populations. It was shown that blood cholesterol level was a determinant of heart attack in these same populations. Population studies like these stimulated an interest in "natural" risk factor experiments that occurred when individuals migrated from one culture to another and from a population with one rate of heart attack to another. Such studies found that the migrants adopted the risks and rates of the country of immigration within a relatively short time. The migrant studies provided additional and confirmatory evidence on risk factor associations, indicated that the genetic nature of one individual was responsive to environmental changes, and showed that risk factor status and risk could be modified.

Early Efforts for Prevention

Although reports were often preliminary and final follow-up analyses often required many additional years, knowledge in these areas was sufficiently developed by complementary investigations to lead to public health pronouncements. The American Heart Association, for example, urged in 1961 that attention be given to controlling fats in the diet to reduce the risk of heart attack. A more emphatic statement followed in 1964.

Much of the risk factor information was gathered together as data for a statement on prevention by the Inter-Society Commission for Heart Disease Prevention. This group of experts was gathered on behalf of the U.S. Department of Health, Education, and Welfare Regional Medical Programs Service to develop guidelines for patients with cardiovascular diseases. A section of the statement was devoted to the primary prevention of atherosclerotic diseases. The initial document, issued in 1970, spoke to the control of risk factors, including dietary modification, obesity, hypertension, and cigarette smoking, to prevent the premature occurrence of heart attack. Meanwhile, an interest arose in the direct demonstration of the benefits of risk factor control because such demonstrations would strengthen the concept of causal relationships and would have immediate practical significance for application to public health and to patient care. The first studies were directed to prospective randomized blinded trials in men who had suffered a heart attack and were at high risk of a second one. The earliest was the Coronary Drug Project, which tested several cholesterol-lowering drugs. The study had been an initiative of the National Advisory Heart Council, and it completed enrollment of more than 8,000 men in June 1969. A preliminary report on the control of lipids was given in 1970. This report was more important for its seminal influence on the design of randomized clinical trials and for findings of drug toxicity than for the demonstration of major therapeutic benefits.

A randomized prospective clinical trial of the effect of diet in preventing initial heart attack, reported in 1969 after 8 years of study by Dayton, Pearce, and associates in a Domiciliary Medical Service of a Veterans' Administration Hospital in Los Angeles, showed a beneficial effect of diet on heart attack that was suggestive but not statistically significant. This and other clinical studies of dietary modification engendered the desire in the early 1960s for a definitive diet-heart clinical trial in free-living men in the open community. A large multicenter National Diet-Heart Study in 1962 undertook a feasibility study for such a trial and examined issues of recruitment, diagnostic criteria, diet prescription, compliance, and metabolic variables. It examined not only common quantitative chemical measures of lipids but also the newly introduced physicochemical measures of lipoproteins. The final report of the study in 1968 showed logistical feasibility, indicated that diet could lower blood lipids, and paved the way for the conduct of a definitive diet-heart trial. The results of the study, however, led to the conclusion that such a trial would be very large and very costly in terms of both money and effort. Its impracticality led a task force to recommend in 1971 that smaller scale well-controlled trials of lipid lowering by diet or by drugs be conducted in high-risk populations. The task force indicated that such studies could be conducted by already initiated Lipid Research Clinics, which were supported and coordinated by the then National Heart and Lung Institute.

The Lipid Research Clinics were a multicenter effort to establish the distribution of normal and abnormal lipid metabolism in the country. As prevalence studies took shape, plans were made to conduct a prospective randomized double-blind clinical trial with a hypocholesterolemic agent for the primary prevention of heart attack among high-risk men.

The Lipid Research Clinics Prevalence Study screened more than 60,000 persons at an initial visit and determined lipoprotein distributions on almost 14,000 of them at a second visit. Similarly, data were acquired on approximately 16,000 children. The data, published in 1980, provided highly standardized, quality-controlled data sets of great value in describing age and sex distributions of triglycerides, cholesterol, and lipoproteins. It was found that plasma cholesterol was somewhat lower than anticipated and that triglycerides were somewhat higher. A decline in the national plasma cholesterol levels during the 1960s appears to have occurred in parallel with dietary fat changes during the same period. Among many other findings, an effect of oral contraceptives to raise plasma cholesterol and triglycerides was reported as well as a beneficial effect of the use of estrogen preparations on death rates among postmenopausal women. Cigarette smoking was found to depress the beneficial high-density lipoprotein (HDL). A long-term follow-up of a portion of the original cohort will provide information on health and disease associations with observational variables already recorded in the study data bank.

The Coronary Primary Prevention Trial, planned in 1971, recruited its first patient in 1973, completed recruitment in 1976, and completed data collection in 1983. Nearly 500,000 middle-aged men were screened, and 3,810 participated in the study. They were otherwise healthy but had plasma cholesterol levels above 265 mg/dl and low-density lipoprotein (LDL) levels above 190 mg/dl. It was a prospective randomized double-blind trial in which all subjects were advised about prudent diet. One-half received cholestyramine, an oral bile acid sequestrant and cholesterol-lowering drug. The trial has shown conclusively that lowering the plasma cholesterol of high-risk men in this manner results in a decreased attack rate and death rate from first heart attack. The data show a high degree of internal consistency. The study did not find clear evidence of toxicity, and the cohort is being followed for any late appearance of adverse effects.

As a consequence of this trial, the concept of a causal role for hypercholesterolemia in human atherosclerosis is greatly strengthened. The finding now requires application in medical practice and public health. The trial, however, was with a drug rather than with diet and was only in men. Nevertheless, the assumption is generally well grounded that a similar qualitative effect could be obtained with diet alone and also in women. There is general agreement in the United States that middle-aged individuals with plasma cholesterol levels above 240 mg/dl should lower them by diet and, if necessary, by medication. This population represents about 25 percent of adults. The ideal, desirable, or attainable levels remain matters of discussion. The average value in the United States today is about 210 mg/dl, and goal values of below 200 mg/dl have been suggested for adults.

Two other findings also may be noted. One is that autopsies reported from several prospective cohort risk factor studies have found that the actual severity of arterial lesions or extent of atherosclerotic plaques is associated with the conventional risk factors, much as clinical coronary heart disease is. The second is that an analysis of the plasma cholesterol data of the 356,000 initial participants in the Multiple Risk Factor Intervention Trial, another Institute-sponsored project of research on intervention against risk factors, found after 6 years of follow-up that there was no threshold of risk but that there was a continuous gradient of risk of heart attack at any level of plasma cholesterol.

It is clear from these investigations that the concern voiced in the 1940s that we lacked substantial evidence to associate hypercholesterolemia and atherosclerotic disease in man has been laid to rest. Not only do we have detailed quantitative information that they are associated, but the influence of the quantity and type of dietary fats on blood lipids and heart attack rates has been documented in humans. Moreover, the circle of argument has been closed by the demonstration that lowering plasma cholesterol levels in high-risk men lowers the risk of atherosclerotic heart disease in a proportionate manner. The NHLBI has used this understanding of the relation of diet, cholesterolemia, atherosclerosis, and heart attack in its recently initiated National Cholesterol Education Program to inform and educate physicians, their health care colleagues, and the public about diet, plasma lipids, and atherosclerotic disease.

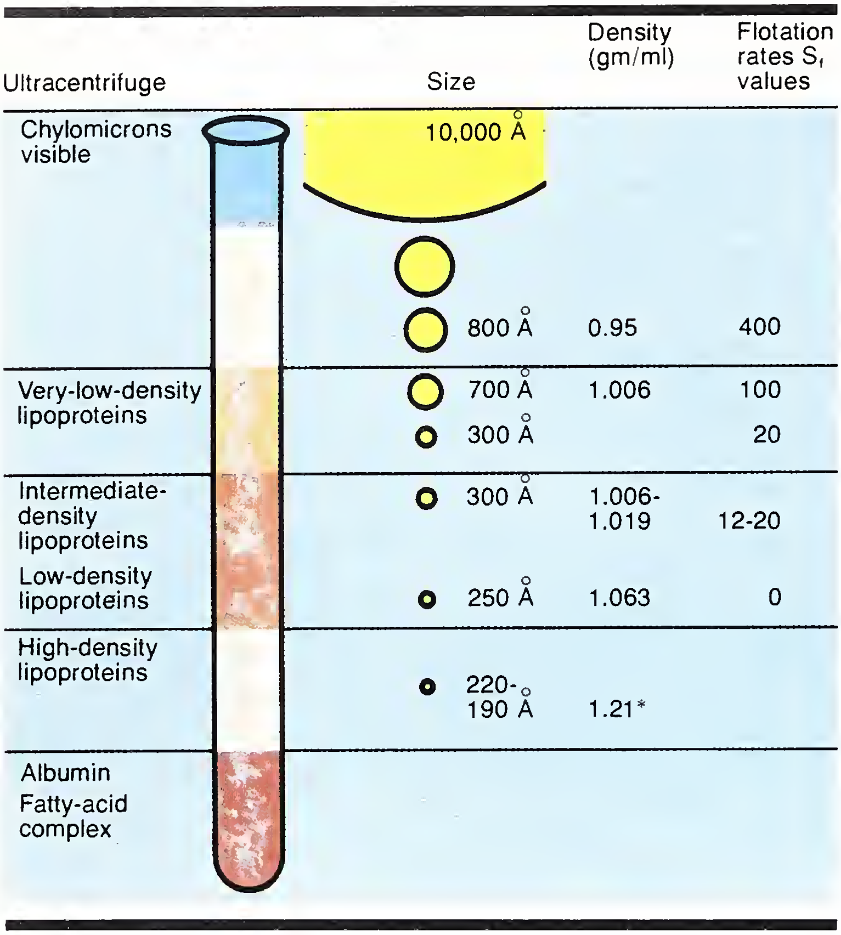
Examining Lipid Metabolism

The cholesterol and cholesterol ester content of normal and atherosclerotic aortas was quantitated chemically in 1910, three years before the first experimental dietary cholesterol model of atherosclerosis was developed in the rabbit. Analysis was extended to other lipids, and in the early 1940s, there was a sound base of quantitative chemical information on bulk samples of normal and diseased arteries.

It was generally accepted that the amount of cholesterol in plaques was too great to have been formed locally in the artery and that it must have come from elsewhere. There was the general view that it came from the lipids of the blood with or without the mediation of some hypothetical arterial injury. This concept was the "infiltration theory" of atherogenesis and, because of the prominence it gave to cholesterol and other fats, it was mainly a lipid theory, although it did not exclude other substances carried in the blood, such as fibrinogen.

Early experiments had shown that growing rats eating a diet free from cholesterol nevertheless increased their total body cholesterol as they grew. It had been shown in 1920 that human infants excreted two to four times as much cholesterol as was contained in their food. Incubated chicken eggs were found to contain more cholesterol than eggs that were not incubated. Obviously, the organism can make cholesterol. In the 1940s, it was demonstrated, with the use of "heavy" water, that the body can convert a very small organic molecule like acetate to cholesterol and that about 13 percent of the hydrogen atoms found in the cholesterol had come from the acetate.

The congruence between the lipid infiltration theory and the emerging information from epidemiologic, clinical, and animal research led to a growth of research on lipid metabolism. Research on the cholesterol metabolic pathway progressed rapidly, drawing on the well-established field of sterol biochemistry. The advent of radioactive tracers also advanced the field rapidly, and the complexities of the origin of plasma cholesterol, studied by means of C^{14}-labeled acetate, were under investigation around 1950. Whether cholesterol was or was not made in situ in the aorta was tested in 1951, and synthesis was found to occur in rabbits and chickens. Subsequent studies, however, showed that this effect was species-specific and did not occur in dogs, rats, or humans. Moreover, tracer experiments in dogs early in the decade had shown the transfer of cholesterol from the plasma to the aorta and, by 1959, the same effect was shown in humans. These experiments also showed that cholesterol was transferred to arteries much faster than it was accumulated and that there must be an efflux. Thus, the infiltration theory emerged as an established concept rather than merely an intellectual idea. Conceptually important work in the mid-1960s refined the idea to indicate that the influx of cholesterol was not a physically passive phenomenon, as the term infiltration might imply, but that the normal intimal tissue presented a barrier or restriction to its influx.



Ultracentrifuge	Size	Density (gm/ml)	Flotation rates S_f values
Chylomicrons visible	10,000 Å		
	800 Å	0.95	400
Very-low-density lipoproteins	700 Å	1.006	100
	300 Å		20
Intermediate-density lipoproteins	300 Å	1.006-1.019	12-20
Low-density lipoproteins	250 Å	1.063	0
High-density lipoproteins	220-190 Å	1.21*	
Albumin Fatty-acid complex			

The ultracentrifugal properties of plasma lipoproteins.

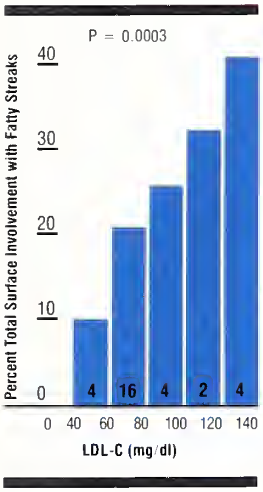
An Important New Method of Analysis

A major revolution in the field of lipid metabolism and atherogenesis occurred when the physical-chemical methods of protein analysis were introduced to qualitate and quantitate the physiological forms in which the water-insoluble fats and cholesterol were carried in the blood. These were methods for the separation of lipid-protein complexes, or macromolecules, and they were based on differences in density or in electrical charge and size. They represented the measurement of biologically valid and relevant forms of molecules called lipoproteins rather than nonbiological solvent-extracted chemical lipids. These new approaches were taken between about 1947 and 1950 in the field of protein chemistry by investigators interested in atherosclerosis. Within 5 to 7 years, papers appeared on such subjects as the distribution and chemical composition of human serum lipoproteins, an enzymatic defect associated with altered lipoprotein metabolism in hyperlipidemia; the chemical composition of four different lipoproteins; the influence of dietary factors on serum lipoprotein concentrations; and the influence of gonadal hormones on serum lipids and lipoproteins. In 1956, a technical group of the National Advisory Heart Council reported an evaluation of serum lipoprotein and cholesterol measurements as predictors of clinical complications of atherosclerosis.

Research continued on the variety of lipoproteins and their origins, fate, and relationships to one another. Metabolic disturbances of an environmental or genetic type or disturbances secondary to other diseases proved illuminating and, as noted above, the protein moieties of lipoproteins came under study. They became known as apoproteins in reports in the late 1950s and mid-1960s. It was felt that study of the molecular structure and function of the apoproteins would lead to more fundamental and informative metabolic issues.

Classifying Lipoproteins

The intramural research laboratories of the then NIH made very important contributions in the late 1960s to a system of classification of the various types of clinical hyperlipemia in terms of lipid and lipoprotein patterns. The classification consisted of five types based on various criteria. Four classes of lipoproteins called chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) had been



The relationship of increasing levels of low-density lipoprotein cholesterol (LDL-C) to increasing fatty streak involvement in children and adolescents in the Bogalusa Heart Study.

isolated by various methods. It was apparent that LDL was a particularly atherogenic lipoprotein and VLDL was not suspect. In particular, rare individuals were found to be genetically homozygous for elevated levels of LDL (homozygous familial Type I disease). This inborn error of metabolism, first described in 1938, is now called familial hypercholesterolemia (FH). Persons with FH have very high levels of LDL cholesterol and suffer premature atherosclerosis and heart attack, even in childhood. Biologically meaningful associations between variations in lipid metabolism and clinical atherosclerotic disease were being drawn, and the laboratory diagnosis of these variations was being put on a firm basis.

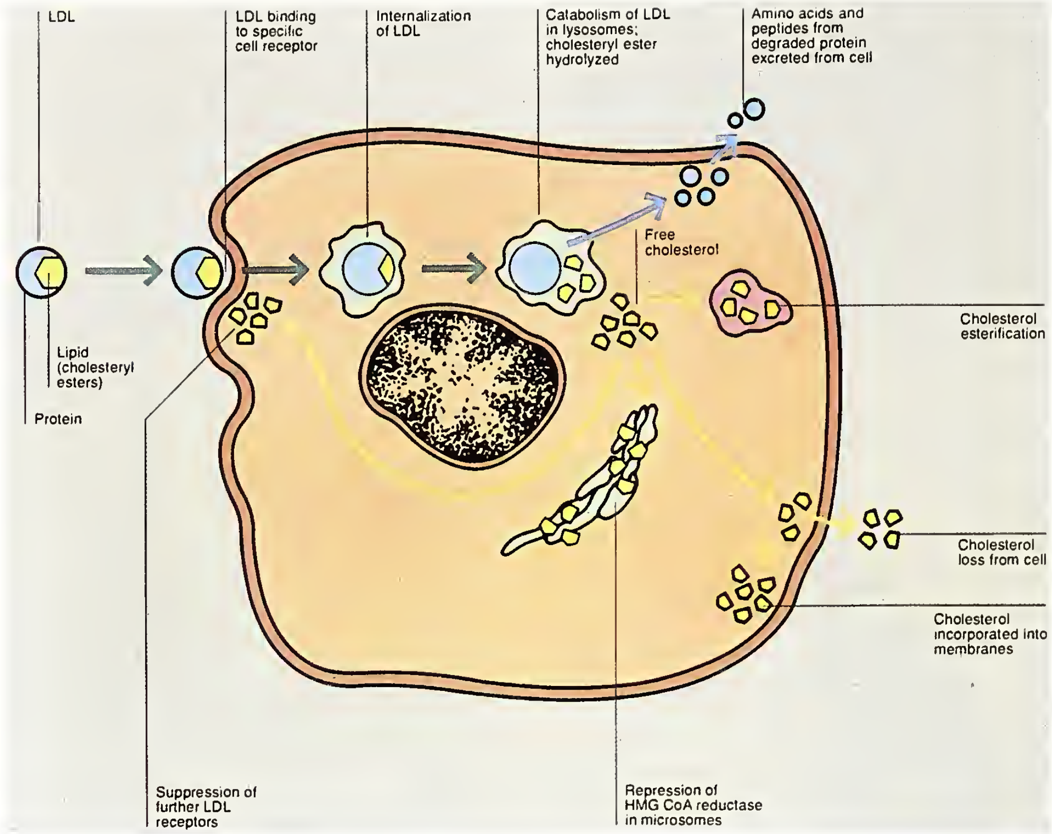
In the early 1960s, the infiltration concept was strengthened further with the use of the immunogenic lipoproteins to generate antibodies, which were then used to identify, by immunohistochemistry, the presence of serum proteins, including beta-lipoprotein (LDL), in the arterial wall. Research in lipid metabolism in the 1970s followed two main paths. One was to gain understanding of the synthesis, degradation, and metabolic interrelationships of the lipoproteins transported in the blood. The other was to study the economy or metabolism of cholesterol at the cellular and molecular levels. In each case, the research was facilitated greatly by the investigation of genetic variants of lipid metabolism in humans, and enormous technical advances that had occurred in the methodology of biochemistry and protein chemistry.

Understanding the Cellular Metabolism of Cholesterol

Cholesterol is a complex four-ring structure that can be made in the body from a simple substrate through a pathway involving at least 30 enzymatic steps. It is not soluble in water. It helps to form the membranes of cells. It is essential to life and to the functioning of the body's cells. Its transport in lipoproteins provides solubility in the blood.

Understanding of the cellular metabolism of cholesterol came from a series of studies that began in an attempt to understand FH. Fibroblasts from the skin of normal patients and of patients with FH were studied in cultures of cells exposed to lipoproteins or deprived of them. The results, measured by a rate-limiting enzyme near the beginning of the cholesterol synthetic pathway (HMG CoA reductase), showed that the presence of cholesterol (lipoprotein) caused normal cells to suppress the enzyme and the relative absence of cholesterol to enhance it. FH cells were unresponsive. LDL was the effective lipoprotein, and it was effective at very low concentrations. Additional experiments, which smuggled cholesterol into FH cells, showed that the enzyme was then suppressed, and it was concluded that the failure noted above must be a genetic fault with the normal mechanism of entry of cholesterol into the cell. The fault was found to be a lack on the cell membrane of a high-affinity receptor to bind and to internalize LDL into the cell. Additional experiments disclosed that receptors for LDL were multidomain glycoproteins that gathered in pits in the cell membrane and then entered the cell where the protein of the bound LDL was digested, its cholesterol esters were hydrolysed, and the receptor was returned to the cell surface to repeat its LDL binding and cell

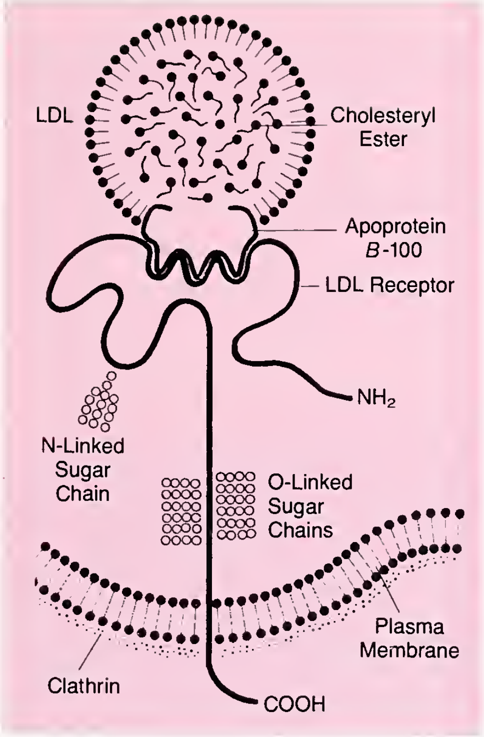
LDL and cholesterol metabolism.

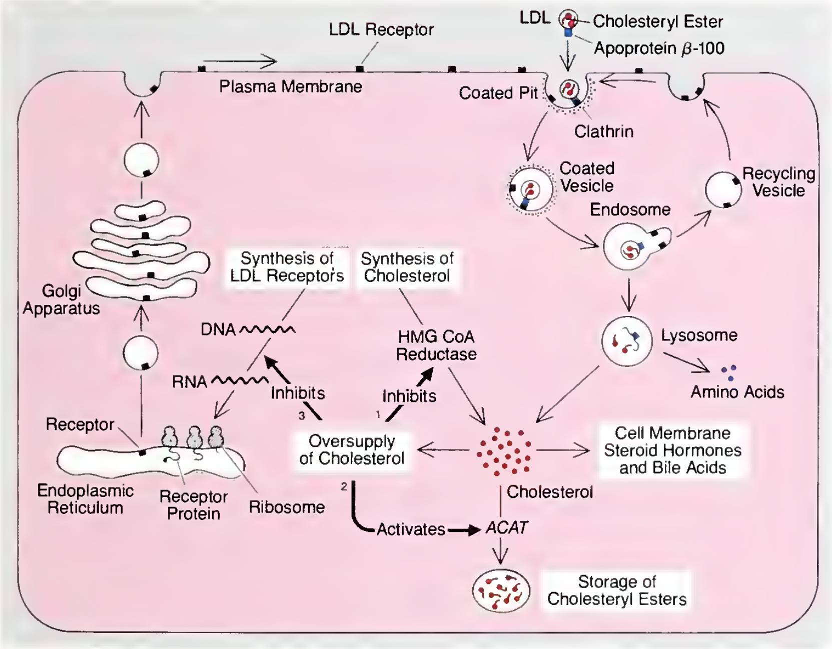

LDL
LDL binding to specific cell receptor
Internalization of LDL
Catabolism of LDL in lysosomes; cholesteryl ester hydrolyzed
Amino acids and peptides from degraded protein excreted from cell
Free cholesterol
Cholesterol esterification
Lipid (cholesteryl esters)
Protein
Cholesterol loss from cell
Cholesterol incorporated into membranes
Suppression of further LDL receptors
Repression of HMG CoA reductase in microsomes

entry cycle about every 10 minutes. The cholesterol delivered to the cell depressed HMG CoA reductase and hence cellular cholesterol synthesis. It also depressed the formation of new receptors. The cholesterol was reesterified for cell use by an enzyme (ACAT) in the form of cholesteryl oleate. It also was found that FH could be a result of different genetic structural defects in the receptor—that is, failure of the receptor to migrate to the cell surface, failure to gather in pits and enter the cell, failure to bind LDL, and failure to form adequate numbers of receptors. By 1985, the structure of the LDL receptor gene was known. It is located on chromosome 19 and is composed of 18 exons and 17 introns. It shows evolutionary conservation of structure or homology with the C9 component of complement, with epithelial growth factor precursor, and with factors IX and X and protein C of the blood-clotting system. The molecular structure of some mutations in the receptor gene have been described.

Understanding the LDL receptor-mediator pathway of cellular cholesterol homeostasis has the most fundamental biological importance since cholesterol is an essential building block of all cell membranes. The understanding also has had a profound influence on our concepts of how cells in atherosclerotic plaques may accumulate lipids and how the levels of circulating or transport lipoprotein may be set. In the first instance, it has turned attention to how various cells, such as liver cells, vascular smooth muscle cells, and macrophages, may utilize specific or nonspecific receptor mechanisms to take up lipoproteins. In the second instance, it has focused on the

LDL receptor present in the plasma membrane of most body cells.





Receptor-mediated uptake of plasma LDL and processing by the cell.

receptor-mediated degradation of lipoproteins and the roles of apoproteins in lipoprotein-receptor interactions. Some receptor mechanisms participate in feedback regulatory systems (LDL), but others do not (macrophage scavenger receptor).

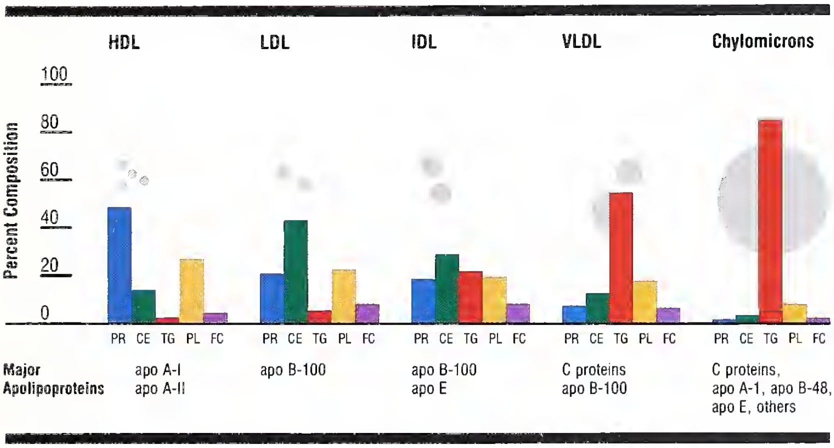
In the 1970s, it was found that labeled LDL was removed from the circulation of patients heterozygous for FH more slowly than from normal people. This finding correlated roughly with receptor deficiency. It was reasoned that approximately two-thirds of LDL removal from the blood is by the LDL receptor mechanism. Later work showed that about 70 percent of the uptake of LDL in the body occurs in the liver, with lesser fractions occurring in other organs.

By the early 1980s, it was clear that a major factor in blood cholesterol (LDL) levels had to be attributed to receptor-mediated LDL degradation balanced against input from dietary absorption and synthesis. Moreover, it was clear that the receptor and HMG CoA reductase pathways offered potential for the treatment of hypercholesterolemia by attempting to increase the number of receptors, by inhibiting HMG CoA reductase, or by both. Comparative studies in various animals and developmental studies in humans of LDL receptors as a homeostatic mechanism also suggested that much lower blood cholesterol levels may be more natural than those experienced by the U.S. population today.

While the complex story of the cellular economy of cholesterol was being elucidated, parallel studies of lipoprotein metabolism and transport in the blood and lymph fluid were being advanced. The levels of the various lipoproteins in the plasma are determined by the dynamic equilibrium between their formation, interconversion, and degradation, and these phenomena were brought under closer scrutiny.

The Role of Apoproteins

Lipoprotein particles are complexes that comprise a core of fat and cholesterol made soluble in the plasma by a surface layer of phospholipids and proteins that are compatible with both fat and water. Lipoprotein particles range in size from the very large chylomicrons to the small high density lipoproteins, and they vary from one another in protein and lipid composition. The smaller species are poor in triglyceride fat, while the larger are rich in fat. The lipoproteins were found to have complex apoprotein composition. Of special interest among the dozen or more that have been identified are: apoprotein B (the critical apoprotein of LDL and VLDL) and apoproteins AI and AII and E in HDL. Apoproteins CI and CII occurring in VLDL were found to be important for the action of lipoprotein lipase and the action of another important enzyme called lecithin cholesterol acyl transferase (LCAT).



Chemical compositions of five major classes of serum lipoproteins, and the distribution of major apolipoproteins among the lipoproteins. The stippled circles represent the approximate relative diameters of the lipoproteins.

In further work on the apoprotein composition of lipoproteins, it has been found that apoproteins are not only essential for solubilizing the lipids for transport in the blood but they also confer on the various lipoproteins precise receptor-mediated interactions with the cells of the liver and other organs and tissues. Thus it has been found that there are two major apoprotein B forms. One, apo-B-48, is a major constituent of chylomicrons from the gut, while apo-B-100 predominates in VLDL formed by the liver and thus predominates in LDL. An additional apoprotein, apo-E, is present on chylomicrons and their remnants and on VLDL. The liver has an apo-B/E receptor that binds LDL and probably IDL (intermediate density lipoprotein), while a distinct E receptor binds chylomicron remnants (see below). The LDL receptor in peripheral tissues binds apo-B. Apoprotein E also has been found to show genetic heterogeneity. Of special note is that apoprotein E is important to the receptor binding in the liver of a form of HDL that arises in the plasma when typical HDL acquires apo-E synthesized by various cells in the body or from other lipoproteins. It appears that this form of HDL can accept cholesterol from body tissues and carry it to the liver for excretion. This mechanism provides a biological explanation for the epidemiologic observation that a relatively raised HDL cholesterol level is a beneficial risk factor protective against heart attack. Recent research has made it clear that the apoproteins (of the lipoproteins) regulate their specific metabolic functions and that they can direct cell surface receptor-lipoprotein interactions.

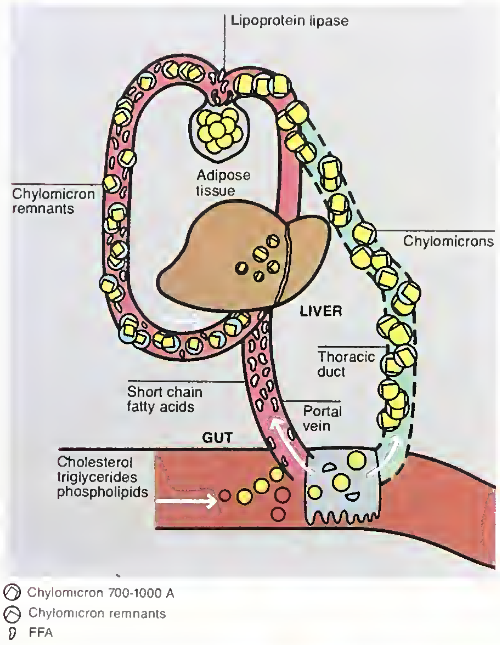
Our current understanding of normal events as enlarged and developed by research over the past decade is that dietary fats are absorbed from the intestine, facilitated by the intestinal synthesis of apo-B-48, and appear as lymph chylomicrons in the blood. There, apo-C and -E are transferred to them from HDL and allow lipoprotein lipase and LCAT to act. The fat-rich chylomicrons also carry cholesterol from the diet, from cholesterol excreted in the bile, and from cholesterol newly synthesized by the gut in the form of cholesterol ester. The lipoprotein lipase digests the fat in the chylomicrons and forms chylomicron remnant particles, which are rapidly removed by the liver through receptor-apoprotein-E binding. Thus, cholesterol and fatty acid energy are supplied to the liver.

Similarly to the intestine, the liver secretes triglyceride and cholesterol-rich particles in the form of VLDL when stimulated by increased concentrations of fatty acids or carbohydrates. They contain apo-B-100, apo-C, and apo-E, and lipoprotein lipase acts to convert them to IDL and LDL. The IDL is removed largely by the liver through apo-B/D receptors.

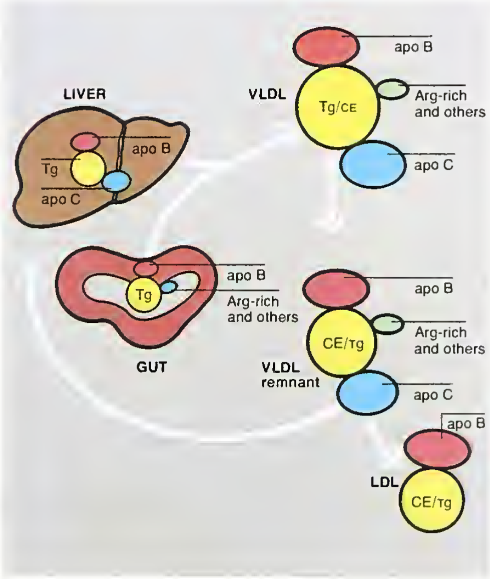
While LDL has been recognized for many years as a preeminent atherogenic protein, there is growing evidence that, in the heterogeneity of the VLDL-IDL cascade, and perhaps also among the chylomicron remnant particles, other important atherogenic species of lipoproteins occur.

LDL is formed primarily from the degradation of VLDL. It is relatively enriched in cholesterol, having lost its fat to digestion by lipoprotein lipase. Its protein portion is almost entirely apo-B. As noted earlier, the degradation of LDL occurs in the liver and peripheral tissues in large part through binding to the high-affinity LDL receptor on the surface of the cell membrane, followed by internalization into the cell and digestion within it. This, in turn, minimizes the cellular synthesis of new cholesterol by inhibiting HMG CoA reductase, reduces the synthesis of new receptors, and increases the esterification of cholesterol by the cells. This process is critical in establishing the homeostasis of the circulating levels of LDL in the blood.

Phases of chylomicron metabolism: (1) intestinal synthesis; (2) hydrolysis of triglyceride in peripheral tissues to form remnants; (3) hepatic removal of remnants.



Synthesis of VLDL and conversion of the LDL remnants to LDL.



Components of HDL originate in the liver, intestine, and plasma. Apo-AI, II, and IV are secreted by the intestine and are part of the chylomicron particle. In the course of degradation of the fatty cores of these particles (chylomicrons and VLDL), an excess of protein, phospholipid, and cholesterol appears as surface material that can be a source of HDL. Additionally, it appears that the liver and intestine secrete particles efficient in cholesterol esters, which are converted to HDL by LCAT. There is considerable heterogeneity in HDL particle size and composition. Typical HDL does not contain apo-E and does not bind to liver cells. However, it can acquire apo-E secreted from various cells or from the surface of other lipoproteins, and it then appears to be an effective carrier or reverse transport mechanism for delivering cholesterol from cells, tissues, and the plasma to other lipoproteins and to the liver for excretion after binding to apo-E receptors on the liver cells.

About three-quarters of the plasma cholesterol is synthesized in the liver and intestine, while about one-quarter is exogenous from the diet. From the dietary point of view, while there are some differences among different mammals, the effect of feeding relatively high fat and cholesterol diets is to produce significant but sometimes subtle changes in plasma lipoproteins of the kind associated with accelerated atherosclerosis. The principal changes are the appearance of HDL with acquired apo-E, a decrease in typical HDL(without apo-E), an increase in LDL, and the occurrence of beta-VLDL, which is a newly formed lipoprotein that may arise from chylomicron remnants or may be newly secreted by the liver under the stimulus of the diet. Beta-VLDL is actively accumulated by macrophages to produce cells with a foamy cytoplasm like those seen in atherosclerotic plaques. It recently has been found that similar but more subtle and heterogeneous changes can occur as transient events shortly after meals. The question of brief but repeated and cumulative atherogenic stimuli from particular species of postprandial lipoproteins within the heterogeneous response to diet is a current research issue.

Recent years have seen the application of the techniques of molecular biology to the study of lipoprotein structure and function. The major apoproteins have been sequenced by analyzing the array of the amino acids that make them up, and this information has been used to detect genetic variants in some of them, for example, in the substitution of single amino acids at specific locations in apo-E. These substitutions affect the receptor binding capacity of apo-E. The most recent apoprotein to have its structure elucidated has been apo-B, a very large apoprotein technically difficult to study. Its structure was elucidated by recombinant DNA techniques. The genes for several of the apoproteins have been located on particular chromosomes, and the gene structure of several has been elucidated.

Forty years of research have progressed from the study of lipids as extracts measured by analytical chemistry to the application of protein chemistry, the study of lipoprotein structure and function in metabolism, the application of molecular biology, and the identification of gene structure and function.

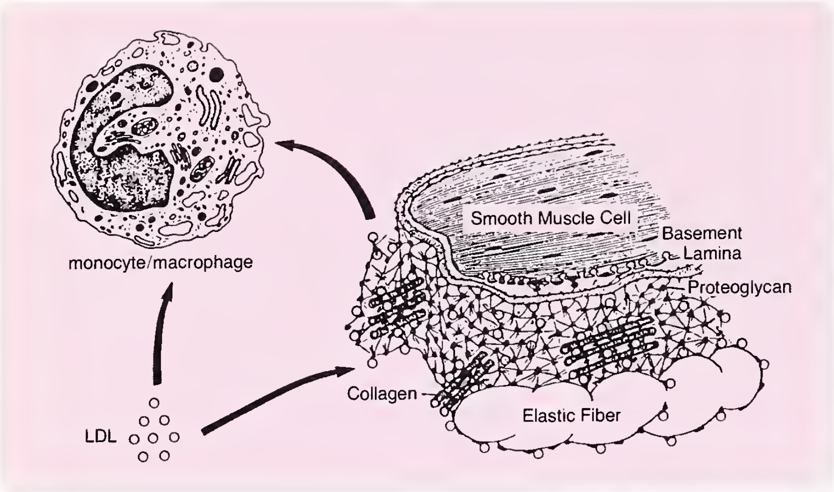
The Development of Atherosclerotic Plaque

The reactions of the arterial wall that comprise arteriosclerosis are at once limited and varied—limited because the reacting artery is composed of only a few kinds of cells and varied because these cells have a range of responses that can be combined in many ways. The arteriosclerotic reaction is localized or focal. It results in an increased volume of tissue, which is space-occupying and is called a plaque. It does not occur at random but is systematic and shows patterns of localization and sequences of occurrence in different arteries. Degenerative changes can be superimposed on its developmental stages. When the plaques are large, they can restrict blood flow and can be the site of arterial blockage when a blood clot or thrombus forms upon them. This capacity for obstruction is the main reason for their clinical importance.

Forty years ago, the variety of human lesions had been well described by conventional pathology and light microscopy. An experimental model in cholesterol and fat-fed rabbits had been described in 1913, and a variety of metabolic experiments of a descriptive type had been performed. Other animal models using chickens, dogs, and nonhuman primates were just being developed. The infiltration lipid hypothesis had been emphasized by the animal research, and an injury-repair modification of it had been carefully argued in 1935. By 1948, the proliferation of cells in experimental plaques had been documented by describing mitotic activity in them. By 1950, the phagocytic activity of macrophage foam cells in experimental plaques had been demonstrated by showing that they could take up small, finely dispersed particles from the blood stream.

The proliferative aspect of plaques in experimental atherosclerosis subsequently was studied in detail by the application of tritiated thymidine as a radioautographic marker for the division of cell nuclei. The technique was introduced to atherosclerosis research in 1962 and subsequently elaborated to study cell population dynamics under a variety of experimental conditions, such as diet or vascular injury and healing. It was shown that endothelial cells, vascular smooth muscle cells, and macrophages could divide and multiply and that the plaque was a hyperplastic lesion.

Plaques are composed not only of an excessive number of cells but also of extracellular materials such as lipids (which are also intracellular), fibrin, and cell products such as collagen, elastin, and jelly-like glycoproteins that fill the spaces between cells and separate them. However, both collagen and glycoprotein chemistry were not well developed in the 1950s and 1960s, and only morphological descriptions could be given. Lipid chemistry was much more advanced, and a detailed account of the several kinds of lipids and their proportions and amounts was possible. Research on plaques up to about 1970 was primarily descriptive and nonquantitative. It sought to understand the variety of lesions and their temporal sequence and the earliest changes in the arteries that could be detected with the microscope. The concept was already well established that it was much more important and perhaps easier to understand the beginnings of plaques than their more advanced, variable, and complex expressions. It was a concept justified on the practical grounds that it would be better



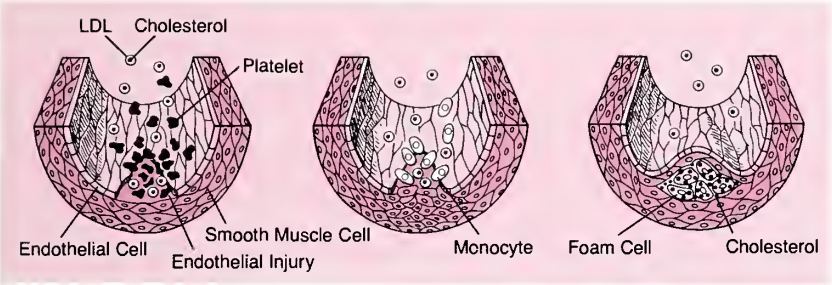
Potential role in extracellular and intracellular lipid accumulation in atherogenesis.

to prevent the initiation and progression of plaques than to seek to cure advanced pathological changes.

Among the important findings prior to 1970 were the realization that blood clot materials such as thrombi and fibrin could elicit a plaque-building reaction from the artery, the identification of arterial smooth muscle cells as major components of plaques (these cells had been regarded as fibrocytes or primitive connective tissue cells), and a large geographic pathological study of the prevalence and extent at autopsy of the simple plaques of childhood (fatty streaks) and of the forms of plaques seen in adults (fibrous and complicated lesions). Toward the latter part of this period, enormous methodological advances were made in the application of the electron microscope and of immunohistochemical and tracer methods to plaques.

A speculation dating from 1852 was restated in 1946 and elaborated in subsequent years—that blood clot materials might encrust the surface of arteries and lead to plaque development by the ingrowth of vascular wall cells into the clot to convert it to scar tissue by normal repair or healing processes. This pathway for the development of plaques is obviously different from the lipid infiltration pathway. Its validity was strengthened by the application to human plaques of immunohistochemical probes for fibrin in 1960 and for blood platelets in 1965. It was found that fibrin occurred in both fatty streaks and adult lesions. Moreover, it could be identified both in the body of plaques and on the lumenal surface, which suggested the operation of both encrustation and infiltration mechanisms. Fibrin was a very frequent finding in human plaques. In contrast, platelet antigens commonly were identified in advanced plaques but were sparse in fatty streaks, which raised doubts about their participation in the early development of plaques. Nevertheless, the frequent participation of blood coagulation proteins and platelets in plaque building was well established by 1970. What was not established and what remains controversial today is whether these elements participate at the start of plaque formation, whether there is a class of plaques that forms exclusively by this pathway, and whether these mechanisms have a role in what is called diffuse intimal thickening of arteries—a thickening some investigators believe provides the tissue substrate for subsequent plaque formation.

The realization that vascular smooth muscle cells were very common in plaques came in about 1960 with improved methods of microscopy. Not only were smooth muscle cells identified in surroundings where they had to be the source for adjacent collagen and glycosaminoglycan formation, but transitional forms of smooth muscle cells to lipid-rich foam cells like the foam cells of macrophage origin were identified by electron microscopy. The production of connective tissue proteins by vascular smooth muscle cells in cell culture was shown by 1971. Such findings also were confirmed in the more advanced lesions of cholesterol-fed experimental animals and led to a period of intense interest in vascular smooth muscle cells and atherogenesis. That monocytes could gather within a few days in focal masses in the aortic intima of cholesterol-fed rabbits was shown in 1957, and their prompt acquisition of lipid droplets and conversion to macrophage foam cells was described. Recent studies utilizing the scanning electron microscope have shown the adherence of monocytes from the blood in groups on arterial endothelium and their migration through the endothelial membrane into the intima, and their conversion there into lipid-containing macrophages as early steps in the experimental initiation of atherosclerotic plaques.



Proposed mechanism for the possible development of an atherosclerotic plaque.

As investigators entered the 1970s, they had evidence that plaques could arise by at least two pathways and were proliferative, that dividing and secretory smooth muscle cells were important components, that monocyte/macrophages participated, and that lipid infiltration and accumulation were hallmarks. However, the relative participation of these various elements was not clear, and there was no substantial information about pathobiological mechanisms.

Research on Plaque Using Autopsy Studies

At the end of the decade, the International Atherosclerosis Project, supported by the then NHI, published a report on the extent of the severity of atherosclerosis of the human aorta and coronary arteries seen at autopsy. The authors reported on findings in over 21,000 autopsies of individuals aged 10 to 69 years. Two-thirds were male; about one-half were white, one-quarter black, and one-quarter Native American. Fourteen countries were represented, of which one was in Europe and the remainder were in the Americas. This study reported on the extent of fatty streaks, fibrous plaques, and complicated lesions seen in these various populations—some of which had high death rates from heart attack, while others did not. While the average extent of atherosclerosis varied in the different populations, the characteristics of the plaques did not seem to differ from one country to another. It was documented also that while the extent of atherosclerosis of the coronary arteries in patients with coronary heart disease could vary considerably, there was an average or "threshold" extent for heart attack that was the same in all countries, regardless of the marked variations in death rates from heart attack between them. A somewhat surprising finding was that the extent of fatty streaks of the aorta and coronary arteries in young persons (10 to 24 years of age) was about the same in countries that had low mortality rates from heart attack as in countries with high rates. Moreover, fatty streaks of the coronary arteries were at least as extensive in females as in males; in fact, they were most extensive in young black women. Since it commonly was held that such fatty streaks were the precursors of adult-type plaques, it was clear that either this view was mistaken or some kind of selective process was at work. It is a problem that is not solved today but recently has come under intensive research.

Another important finding during this period came from autopsies on young men killed in the Korean and Vietnam wars. The studies were not extensive, but they documented clearly that young men in their late teens and twenties could have raised fibrous plaques in their coronary arteries that narrowed the arteries appreciably. A minority of individuals at this age already had adult-type plaques. The findings were profoundly influential for those interested in preventive medicine since they implied a still more youthful onset of atherosclerosis and made it a "pediatric disease." The questions of the prevalence and development of such lesions in youth remain unresolved, but they have come under study recently. It has been found that an occasional individual as young as 15 years of age may show well-developed coronary lesions in a diffusely thickened intima that are transitional in appearance between fatty streaks and the typical raised fibrofatty lesions of adult type.

Using Cell Biology To Study Plaque

Although the study of plaque was largely descriptive in morphological or biochemical terms until about 1970, important hypotheses existed, and they could be tested in terms of descriptive variables. In the 1970s, cell and tissue culture methods using sensitive and specific biochemical micromethods were applied, especially methods for the identification of proteins. Research in atherosclerosis adopted the methods and science base of cell biology and became reductionist in character and, for the first time, methods to study the detailed mechanisms of components of plaque development became readily available. Descriptive research became less common, and research on living animals was reduced in favor of cell culture studies, which offered relatively simple systems for study. One landmark study of mechanisms that already has been mentioned was the discovery of the cell surface high-affinity LDL receptor. Elucidation of the economy of cholesterol metabolism at the cellular and molecular levels has been profoundly informative and influential. A second important contribution has come from the identification of a growth factor carried by blood platelets that stimulates vascular smooth muscle cells to divide.

Platelet-Derived Growth Factor and Atherogenesis

It was observed in cultures of vascular smooth muscle cells that when platelets were removed from the serum used in the culture medium, the cells did not multiply as usual. Restoration of platelet-rich medium stimulated cell division. This observation was verified indirectly in intact animals subjected to an atherogenic stimulus in which platelet activity was suppressed. In one model in which a large area of arterial endothelium was denuded mechanically, platelet suppression inhibited the florid proliferation of smooth muscle cells that ordinarily follows such vascular injury. Subsequent work followed two directions: to seek further understanding at the molecular level and to study experimental variations in living animals to ascertain how the phenomenon participated in atherogenesis.

Research at the molecular level purified the factor, determined its molecular weight, and provided its partial structural sequence of amino acids. A specific receptor for the platelet-derived growth factor (PDGF) is found on the smooth muscle cell surface. A finding of great interest was that the PDGF amino acid sequence and that of a transforming protein derived from an oncogene—under parallel study in the field of cancer biology— contained extensive domains of similarity; the homology was 87 percent. There was congruence between this normal growth factor and a growth factor concerned with tumor growth. The discovery of PDGF opened the field of research in atherosclerosis to the concept of factors that could provide signals for growth and cell differentiation from one type of cell to another (paracrine) or by a cell to itself (autocrine). Other cells of the artery wall were found to form growth factors and to have receptors for them as well as receptors for growth-modulating factors such as insulin or other hormones.

The elucidation of the role of the LDL receptor in cellular and systemic lipoprotein metabolism opened new horizons in atherosclerosis research concerning receptor-mediated processes and scavenger pathways. This information could explain mechanistically the old observations about hyperlipidemia and about foam cell phagocytosis in plaques. The discovery of PDGF also provided a mechanistic explanation for the old observation of mitotic activity in plaques and forced the application of the science base of the biology of cell growth and differentiation to research on atherogenesis.

An immediate consequence of the discovery of PDGF was a reformulation of the injury-repair modification of the lipid infiltration hypothesis of atherogenesis, which in effect linked it with the thrombotic encrustation hypothesis. It was speculated that injury to endothelial cells by agents known to damage them led to their loss in patches or small numbers. The loss of endothelium then permitted the adhesion and aggregation of platelets, local release of PDGF, and stimulation of proliferation of a focal group of smooth muscle cells to form a cell-rich plaque into which lipoproteins could infiltrate and accumulate. It was postulated that this process could be either minimal or marked, transient or repetitive, and cumulative without permanent effect or atherogenic. Thus, it offered a means of reconciling the two major theories of atherogenesis and allowed a mechanism by which platelet incrustation could initiate plaque development. The PDGF also provided a mechanistic explanation for the conversion of well-developed thrombi or blood clots to plaques.

Systematic exploration of this concept in living animals has resulted in considerable uncertainty regarding its relevance to atherogenesis. While the importance of PDGF as a stimulus to proliferation has been shown in certain experiments (see above) by platelet suppression, experiments that were focused on endothelial cell loss cast doubt on the concept. It generally has not been possible to show the patchy loss of endothelium that was postulated, although it should be noted that human arteries have not had adequate study. Moreover, in experiments in which endothelial denudation has been systematically caused in rather precise linear or surface area patterns, the postulated proliferation of smooth muscle cells has not occurred, and the integrity of the endothelial membrane has been restored promptly. A recent experiment has found that when nonhuman primates are fed an atherogenic diet and plaques begin to develop, small retraction defects, or microulcers, occur in the endothelium over plaques after several months. The location and timing of these microscopic defects where endothelium is lost are moderately precise. Subendothelial collagen, fibrils, and macrophages are exposed at the base of the endothelial defects to which the blood platelets adhere. It is postulated that PDGF can then promote proliferation of vascular muscle cells at such foci and that they contribute to atherogenesis initiated by lipid infiltration in this manner. Recent research has discovered that all of the cells involved with plaque building (endothelium, muscle cells, monocyte/macrophages, and platelets) can release various growth factors and chemoattractants for the migration of monocytes and vascular muscle cells. Consequently, it is possible to postulate a complex system of responses to atherogenic stimuli (injuries) in which these substances interact in different ways, both paracrine and autocrine, but with the final outcome of atherogenesis due to their interplay.

Research on PDGF provided a rationale relative to atherogenesis for research on a group of fatty acid derivatives that had been discovered earlier. These substances are called prostanoids and can be formed by many cells, including platelets and endothelial cells. They are powerful messengers between cells, which act briefly, at short range, and in minute amounts. In the present context, they can cause platelets to clump or disperse and cause vascular smooth muscle cells to contract or relax. In particular, it has been found that platelets release a substance called thromboxane A-2, which contracts vascular muscle cells and aggregates platelets into masses. In contrast, the endothelium and vascular muscle cells form and release locally a related but opposing substance called prostacyclin, which relaxes muscle cells and opposes platelet aggregation. Thus, molecular mechanisms of special interest in atherogenesis have been found that may balance these platelet-endothelium interactions. These hemostatic substances have attracted great interest in the areas of plaque formation and arterial thrombosis and spasm, particularly since their formation can be modified by diet and drugs. Nevertheless, additional research will be necessary to elucidate their roles in atherosclerosis and its complications.

The Phenomenon of Plaque Regression

In 1928, Antischkow conducted experiments to study the regression of the plaques of dietary atherosclerosis in the rabbit. It was found that the mobilization of lipid and cholesterol from well-developed fatty plaques was very slow after the removal of cholesterol from the diets of the animals. Some lipid was still present a year and one-half later, and it took 2 to 3 years to obtain lipid-free plaques. The plaques showed excessive intimal thickening and fibrosis.

The subject remained largely unstudied until the late 1940s and 1950s, when descriptive experiments were done with dogs, chickens, and other animals. In general, the studies found that regression occurred in the sense of diminished content of lipid in plaques but that a residuum or even an increase of collagen and matrix remained as a fibrous plaque. An exception to this finding were small and early fatty streaks, which appeared to regress, leaving only minimal intimal thickening. An original observation of this type was a report that the small fatty streaks found in the ascending aorta of suckling rabbits disappeared after they were weaned.

Since experiments dealing with the regression of chronic disease take several years to complete, they are not numerous. However, an adequate number have been done to confirm the earlier observations. In a recent study, an experimental design was used in which nonhuman primates were caused to have atherosclerosis at relatively high levels of cholesterol so that some animals had cholesterol levels about 200 mgm/dl while others were about 300 mgm/dl. These values were maintained over periods of 19 months and also 48 months. It was found that chronicity of induction of atherosclerosis did not affect regression and that while some regression and also some progression occurred in groups of animals maintained at either 200 or 300 mgm/dl of serum cholesterol, considerably more regression and less progression occurred among the group maintained at 200 mgm/dl. The most regression tended to be in animals with total plasma cholesterol to HDL cholesterol ratios of less than 4.5.

At present, nothing is known about the cellular and molecular mechanisms of regression. It is a natural phenomenon that occurs slowly and partially when the atherogenic stimulus is withdrawn.

The concept of plaque regression has different meanings in different contexts. For example, it may mean a change in size (plaque volume), a change that increases the functional vascular lumen, a change to a plaque profile with less tendency to hemodynamic turbulence, a less thrombogenic surface (or more normal endothelial function of any type), a change in cellular or biochemical composition so that some component reverts toward normal, and even dilation or enlargement of the host vessel. All of these meanings may have validity in different circumstances. Most investigations have measured change in plaque size, biochemical content, and lumen size.

It was encouraging to find in animals that lipids would leave the intima even though scarred plaques remained, and investigators sought evidence of regression in humans. A critical review in 1984 found that regression, diagnosed by sequential angiographs, had been reported in over 100 patients by different authors. The review also noted that certain autopsy studies performed in war-time Europe when food was in very short supply found less adult-type atherosclerosis than usual. Whether this was because of regression or lack of progression is, of course, uncertain. The reviewer comments that two anatomical forms of regression may occur. One would involve a loss of plaque substance, as in the animal experiments; the other would involve lysis or resolution of clots and thrombi on the plaque surface. Current noninvasive techniques for visualizing arteries together with fiberoptic inspection of the arteries from the inside may help to resolve these issues. Moreover, certain experiments in cholesterol-lowering supported by the NHLBI may go far in further documenting the circumstances and occurrence of regression in humans.

Current Research Directions

The importance of the new information that came from the application of the techniques and concepts of cellular and molecular biology to the biology of the artery wall and its constituents during the 1970s has led to an emphasis on these research methods in the 1980s. Today, most of the research in the field of atherogenesis deals with the study of simple biological systems in isolated cultures of the cells that are found in arteries. For example, it has been found that the endothelial cell is a complex metabolic and physiological cell. At least 14 functions are now ascribed to it. Among them, roles in vascular smooth muscle cell differentiation, angiogenesis, selective transendothelial transport of large protein molecules, modification of LDL to render it suitable for accumulation by monocyte/macrophages, regulation of clot formation and clot lysis, processing of vasoactive molecules, interaction with blood monocytes, and participation in immunological reactions may all be relevant to one or another aspect of atherogenesis. Because of the many functional roles of endothelium, the concept of endothelial injury has been changed from that of cell death and desquamation to much more subtle concepts of failed or partially failed functions.

Similarly, a vast amount of information is emerging about the various properties of monocyte/macrophage/foam cells. For example, these cells can secrete growth factors, secrete lipoprotein lipase to digest triglycerides extracellularly in the artery wall, secrete chemoattractant factors, accumulate lipid both by scavenger and specific receptor binding pathways, and display many other activities germane to inflammation and repair. Recent work with the monocyte/macrophage gives substance to earlier reports that atherosclerosis expressed features of inflammation. These are among the most versatile of cells, and they may display both putatively beneficial or harmful effects. Whether they migrate out of plaques as well as migrate into the artery wall remains to be shown.

Vascular muscle cells, too, have many functional properties. They secrete the complex fibrillar and gelatinous matrix that makes up a large part of the extracellular material in plaques. They also have the capacity to store lipid and to be converted into foam cells that superficially resemble those derived from monocyte/macrophages. Growth and chemotactic factors also have been identified. Recent findings have described the ability of vascular contractile muscle cells to experience up to about four to six cell divisions, at which point they cease to proliferate in response to stimuli and enter into a secretory noncontractile phase. The appearance or phenotype of the two types of muscle cells is different and so is their function. The secretory type is active in the production of the fibrillar and gelatinous matrix that forms a large part of plaque substance. Matrix components have been found to modulate cell growth and differentiation.

Much is being learned about the capabilities of the cells of the artery wall, and it is apparent that these cells can influence each other by the secretion of signal or communication substances. The study of intercellular signaling or "cross talk" marks a vital turning point in the reductionist analysis of vascular biology and atherogenesis for it is the beginning of investigations that seek to resynthesize phenomena, once studied in isolation, into more complex organizational systems of greater biomedical significance.

Decades of Progress

The past four decades have witnessed progress in understanding atherosclerosis that could not have been imagined when the NHLBI was founded. Progress has been immense, and it has both basic and applied aspects.

While different observers of this progress might reach different judgments or select different examples, it is likely that most would agree that two of the four decades were remarkable for their seminal influence and research findings. The first was the decade of the 1950s, when the associations between hyperlipidemia and atherogenesis in humans were measured and established. This was also the decade in which the phenomena of lipid metabolism and transport were measured, not by simple nonphysiological analytic chemistry of extracted lipids but by measurement of the natural physiological transport forms of lipoproteins by the methods of protein chemistry. Progress in these two areas has been enormously informative and consequential.

The second was the decade of the 1970s, when the techniques of cellular and molecular biology were applied to atherogenesis. Two research findings were particularly informative. One was the discovery of the LDL-cell surface receptor interaction and the elucidation of the cholesterol economy of the cell. The other was the discovery of PDGF and its influence on the hyperplastic aspects of plaques and its opening of unperceived horizons in cellular organizational crosstalk and the behavior of systems of cells. Research of this decade also has been immensely informative and stimulating. Current research derives, and indeed research in the near future will continue to derive, from the strong influences of the 1950s and 1970s.

Much has been learned, and some of it already has found practical application. It is now possible to draw schematic diagrams to show how lipids, thrombosis, and vascular cells can interact mechanistically to produce atherosclerosis. Such diagrams have deficiencies, which will be corrected by future research, but they also have much validity. Atherogenesis is a very variable process with a wide range of expression. It will be necessary to learn in the future the proportionate and interdependent roles of the many and various biological phenomena in the production and prevention of atherosclerotic disease. From the practical point of view, the risk factors for atherosclerotic disease have been characterized, treatments are in hand, and prevention through public and physician education has been initiated.

Gardner C. McMillan, M.D.

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Parsley

Sage

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Follow your doctor's recommended exercise program regularly.

Take your high blood pressure pills every day.

Control your weight.
If the pounds creep up, so can your blood pressure.

High Blood Pressure. Treat Yourself to Life.

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Progress Against High Blood Pressure: A Medical, Scientific, and Public Success Story

The campaign against high blood pressure, "the silent killer," is being conducted on multiple fronts, from research on fundamental mechanisms to clinical and lifestyle treatment strategies.

The story of efforts to prevent and control hypertension or high blood pressure is an exciting chapter in medical history. It is a story that is still evolving and that promises to provide important contributions to science, public health, and individual well-being for years to come.

Four decades ago, hypertension was viewed as a clinical problem that afflicted only a limited number of patients. It was associated with a high mortality rate due to either stroke, heart disease, or kidney failure. Antihypertensive drugs had not been developed, and the only therapeutic intervention available was nonpharmacologic, consisting of severe sodium restriction. Although this intervention proved to be helpful in a significant number of cases, it also was often insufficient, and many of the patients who had high blood pressure died. It was recognized that, in some of these patients, hypertension was due to disease of the kidneys and that disturbances of the adrenal glands also could lead to hypertension. The majority of patients with hypertension, however, were characterized as having primary or essential hypertension, meaning the mechanism accounting for the rise in blood pressure was unknown.

Early Epidemiologic Studies

While clinicians were dealing with relatively few patients with severely elevated blood pressure, actuarial data had been accumulating on large numbers of people that suggested a direct association between elevated blood pressure and an increased risk of dying prematurely from cardiovascular disease. In the late 1940s, the then National Heart Institute initiated the Framingham Heart Study. Over the course of the next two decades, data from this study, as well as from other epidemiological studies, confirmed the association observed in life insurance statistics between elevated blood pressure and increased cardiovascular risk. Figure 1, which is based on data from the Framingham Heart Study, graphically illustrates this point. It can be seen that people at the extreme right-hand side of the blood pressure distribution, those with the highest blood pressures, have the greatest probability of dying prematurely. These were the patients that clinicians were observing in the 1940s, oftentimes dying of stroke, heart disease, or kidney failure brought about by severe hypertension.

What the actuarial data and the epidemiologic data, particularly the Framingham data, have shown us, is that hypertension is a disease that produces clinical morbidity

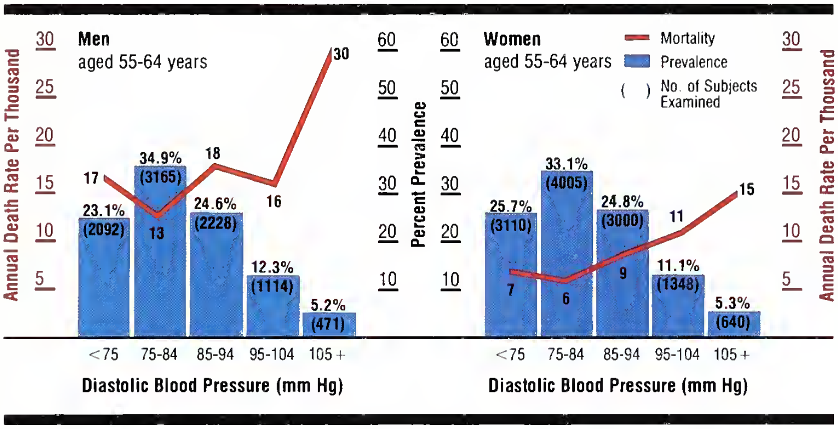


Figure 1.

Data from the Framingham Heart Study illustrate, in women and men aged 55 to 64 years, the direct relationship between diastolic blood pressure and mortality. As the level of blood pressure increases, so also does mortality.

and mortality at the extreme end of the blood pressure distribution. It also is a risk factor, meaning that any elevation of blood pressure, even when mild and asymptomatic, is associated with an increased probability of premature cardiovascular morbidity and mortality.

High blood pressure is found worldwide, although its distribution is oftentimes confined to industrialized nations. Indeed, comparative epidemiological studies suggest that high blood pressure may not be a biologically-determined risk factor since it does not seem to afflict people living in nonindustrialized societies. Why these people tend not to have high blood pressure, while citizens of industrialized societies do, is not entirely clear. There are, however, several hypotheses to account for this observation since hypertensive societies are found to have higher salt intake, lower potassium intake, higher saturated fat intake, are more obese, are more sedentary, and are subjected to a different array of psychosocial stressors.

Antihypertensive Drug Treatment

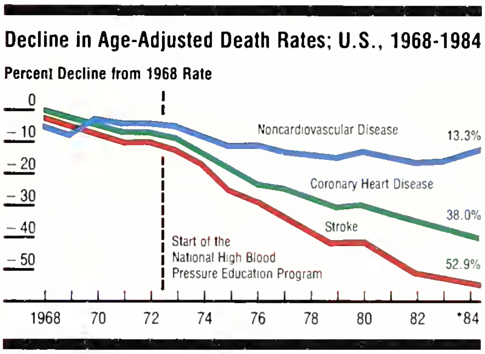
While population-based research was providing insights suggesting that high blood pressure was both a disease and a risk factor, progress was being made in its treatment. Between 1950 and 1952, three antihypertensive drugs became available. These were hexamethonium, a ganglionic blocker, hydralazine, a drug that dilates blood vessels, and reserpine, a rauwolfia alkaloid that interrupts the sympathetic nervous system control of blood pressure. Although these drugs were helpful, especially compared to no drugs at all, the first really impressive breakthrough in the pharmacologic treatment of hypertension came with the introduction of the thiazide diuretics in 1958. These drugs not only reduced blood pressure by themselves but also helped other antihypertensive agents to work more effectively.

Along with these drugs came case series in the clinical literature suggesting that drug treatment of hypertension was associated with a reduction in blood pressure and with more favorable clinical outcomes. In order to test systematically the efficacy of antihypertensive drug treatment, the Veterans' Administration Study Group in 1964 randomized 523 patients with varying degrees of high blood pressure to a placebo or to one of two drug regimens (diuretic plus reserpine or diuretic plus hydralazine). The reports of these trials were published between 1967 and 1972, and established clearly that the treatment of patients with diastolic blood pressures in excess of 104 mm of mercury was associated with a substantial reduction in cardiovascular risk, especially that due to stroke. The benefits were not statistically significant in terms of prevention of heart attacks, however. The results of this trial greatly advanced our concept of the need to treat large numbers of patients with hypertension rather than only a limited number of patients, many of whose deaths were imminent.

The 1970s witnessed the introduction of additional antihypertensive drugs as well as several more clinical trials, including the NHLBI-sponsored Hypertension, Detection, and Follow-up Program trial, the purpose of which was to determine whether mortality related to hypertension could be reduced by using a systematic approach to treatment of elevated blood pressure. The results of these trials suggested that treatment of mild hypertension is associated with decreased mortality and morbidity and decreased progression of disease, but that the benefits are not as dramatic in comparison to the treatment of more severe forms of high blood pressure.

Figure 2.

Age-adjusted decreases in mortality in noncardiovascular disease, coronary heart disease, and stroke and their temporal relationship to the implementation of the National High Blood Pressure Education Program.



*Provisional Rates/NHLBI Estimates
Source Mortality statistics from NCHS and NHLBI estimates

In spite of the high prevalence of the disease, the serious clinical complications of high blood pressure, and the knowledge that antihypertensive drug therapy could reduce significantly hypertension-related morbidity and mortality, data in 1972 from the first National Health and Nutrition Examination Survey (NHANES I) indicated that only half of the subjects who had high blood pressure were aware of it, only half of the aware subjects were being treated, and of the treated subjects, only half had blood pressure under control (blood pressure less than 160/95 mm Hg). In the 1970s, the issue of benefit versus risk in the treatment of patients with mild hypertension had not been addressed fully. But, because of the high prevalence of the disease, the known efficacy of treatment of the more severe forms of hypertension, and the seemingly low levels of patient awareness of the disease and lack of appropriate blood pressure control, Health, Education and Welfare Secretary Elliot Richardson in 1972 established the National High Blood Pressure Education Program (NHBPEP). This ongoing program, administered by the NHLBI, is charged with promoting the detection, evaluation, treatment, and control of high blood pressure through mechanisms designed to enhance awareness and knowledge of high blood pressure on the part of consumers and patients, as well as through education of physicians and other health care providers. Indeed, the Institute takes great pride in this program for, since its inception, age-adjusted stroke mortality in the United States has declined by more than 50 percent (Figure 2). Although there are probably several factors contributing to this decline, the advocacy on the part of the NHBPEP for aggressive control of blood pressure would appear to be a major factor.

Contemporary Approach to Hypertension Research and Blood Pressure Control

Using national probability data collected by the National Center for Health Statistics, the Institute has estimated that hypertension affects 29 percent of the adult population or 58 million Americans. The

BASIC RESEARCH		APPLIED RESEARCH	CLINICAL RESEARCH	CLINICAL TRIALS	DEMONSTRATION AND EDUCATION RESEARCH	TECHNOLOGY TRANSFER	CLINICAL PRACTICE/ PUBLIC
Genetics • classical • molecular biology **Neurobiology** • brain • neurotransmitters • CNS • PNS **Endocrinology** • renin • angiotensin • adrenal hormones • prostaglandins • kallikrein-kinin	**Physiology** • membrane physiology including NA$^+$, K$^+$, & Ca^{++} transport mechanisms • cardiovascular hemodynamics • vasculature • microcirculation **Nephrology** • intrarenal physiology and pathophysiology **Pharmacology** **Pathology**	**Drugs** **DxTests** **Technology** • auto BP devices • infusion pumps	**Descriptive studies** **Hypertension in the young** **Hypertension in the elderly** **Environmental factors** • nutrition • psychosocial factors • genetic environmental interactions **Epidemiology**	**Efficacy of preventive, diagnostic and therapeutic interventions**	**Effectiveness of preventive, diagnostic and therapeutic interventions**		

Figure 3.

The NHLBI addresses a full spectrum of needs and opportunities in its hypertension program.

estimated prevalence in the black population is even higher (38 percent), and older Americans have a higher prevalence of hypertension than younger Americans. In terms of clinical classification of the different types of high blood pressure, our current understanding suggests that 90 to 95 percent of patients have primary or essential hypertension and 5 to 10 percent have secondary forms of high blood pressure. This latter category is comprised of patients whose high blood pressure is a consequence of structural abnormalities within the aorta or renal arteries, or of diseases of either the kidneys or adrenal glands. Primary or essential hypertension, however, remains the empiric term used to describe blood pressure elevated for reasons that are not apparent.

The Institute has planned and implemented a multifaceted approach to the study of hypertension. This approach includes not only research but also comprehensive efforts to transfer the findings of research into clinical management of hypertension. Figure 3 depicts the NHBLI's acknowledgement of the place basic research occupies in the concerted effort to gain better understanding of the regulation of blood pressure and the etiology of pathologic hypertensive states. A variety of disciplines, as indicated in the figure, are utilized. These include, but are not limited to, genetics, neurobiology, endocrinology, physiology, nephrology, pharmacology, and pathology.

Modern research tools of molecular and cellular biology have been utilized to provide new knowledge with potential implications for the regulation of blood pressure and the potential prevention of hypertension. For example, it is now known that the heart, in addition to being a pump, also functions as an endocrine gland. It secretes a potent hormone called atrial natriuretic peptide (Figure 4). This hormone, which is currently the subject of several NHLBI-supported studies, has been found to induce diuresis and also cause relaxation of blood vessels resulting in a fall in blood pressure. Determining the precise role that this plays in hypertension is a major focus of current research investigations.

Basic research, however promising, is not always sufficient by itself. There is a need for applied research to develop new ways of using basic research results to achieve specific practical goals. Similarly, clinical research is very important. This is a focal point in the Institute's overall approach to hypertension because clinical insights direct investigators to the laboratory to determine more precisely the mechanisms underlying changes in blood pressure. Alternatively, clinical research can propel researchers forward to evaluate, under controlled circumstances, the efficacy of preventive, diagnostic, and therapeutic interventions.

In the applied and clinical realms of therapy, new and better drugs have been developed and have been quite helpful in controlling high blood pressure in large numbers of subjects. Recall, however, that the earliest form of antihypertensive treatment was nonpharmacologic, consisting of dietary sodium restriction. It is therefore of interest that, in recent years, there has been a renewed interest in nonpharmacologic treatment of high blood pressure for a variety of reasons. First, the lower the blood pressure among patients selected for drug treatment of high blood pressure, the lower the benefit-to-risk ratio. Since these patients are at lower risk initially, any side effects of the drugs loom larger in the decision to treat the high blood pressure. Because of the side effects of certain drugs, clinicians have become increasingly interested in trying to optimize the use of nonpharmacologic methods in the treatment of high blood pressure as a way to avoid, or at least reduce, pharmacologic therapy. Another factor contributing to the renewed interest in nonpharmacologic intervention is the realization that several dietary constituents, individually or in concert, appear to affect the blood pressure and, in some cases, may be involved in the pathogenesis of hypertension. It makes good clinical sense, therefore, to modify dietary constituents that may be involved in the genesis of high blood pressure in particular patients and, at the same time, avoid exposing them needlessly to possible untoward effects of antihypertensive drug therapy. These attempts to study and optimize nonpharmacologic treatment strategies are complementary to the sound therapeutic principles of drug treatment of high blood pressure, especially as the blood pressure rises to higher levels.

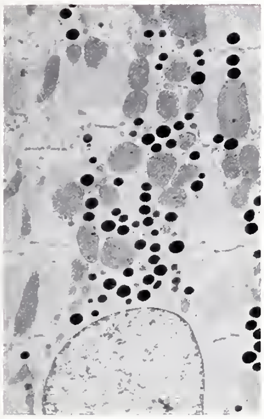
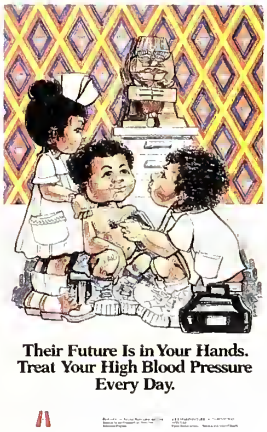


Figure 4.
Atrial granules (black substances) in the rat heart. Atrial natriuretic factor, a hormone present in atrial granules in certain human heart cells, acts to promote kidney function, relax smooth muscle, and lower blood pressure (original magnification x 18,600).

The scientific base underlying the use of nonpharmacologic interventions in the treatment of high blood pressure is not as robust as one would like to see. Recently, however, largely through NHLBI-stimulated mechanisms, more research is being focused on these modalities. Current strategies under investigation include: weight reduction in the obese; sodium restriction; potassium, calcium, and magnesium supplementation; alcohol restriction; increasing the ratio of polyunsaturated to saturated fats in the diet; exercise; and a variety of biobehavioral interventions, including relaxation therapies and biofeedback.

It has become apparent over the years that even when research leads to interventions that appear to be efficacious, that is, they work under ideal circumstances, they will not necessarily be equally effective in the real world. Therefore, the Institute has been sponsoring demonstration and education research that tests the effectiveness of interventions designed to promote health or prevent disease in defined populations. The interventions selected for such testing are ordinarily those that already have been found to be efficacious in other studies and include, but are not limited to, education strategies and modifications in health care and health-related practices. Examples of such demonstration and education research are the NHLBI-sponsored worksite projects, many of which have shown that getting patients to participate in worksite programs of blood pressure monitoring enhances the overall control of blood pressure, not just in individual patients, but in large groups of people, in this instance, those united by a common employer.


Their Future Is in Your Hands.
Treat Your High Blood Pressure
Every Day.

Poster from the National High Blood Pressure Education Program conveys a message to patients with hypertension.

National High Blood Pressure Education Program

Finally, when as a result of basic, applied, clinical, or demonstration and education research, information is ready to be translated to clinical practice, or for the public health, the Institute utilizes the aforementioned National High Blood Pressure Education Program. This program represents a coalition of 15 Federal agencies, 150 national organizations, 50 state health departments, 2000 community agencies, and a variety of other groups. Led by the NHLBI, key representatives from the coalition have formed the NHBPEP Coordinating Committee. This Committee has taken responsibility for coordinating technology transfer to assure that the products of research, when ready for application to the clinical care of patients, are assisted systematically in the transition from research to clinical practice. The use of an orderly consensus process is one of the key strategies employed by the NHBPEP Coordinating Committee. This very productive committee has published numerous scientific documents on the detection, evaluation, and control of high blood pressure targeted to a broad audience including physicians, nurses, dietitians and high blood pressure program coordinators. Other functions of the NHBPEP center around educational activities directed toward consumers and patients. A multimedia approach is utilized including printed brochures, posters, billboards, radio spots, and television public service announcements. Modern-day public relations techniques are incorporated into these educational messages.

Increasing Public Knowledge

Although much work remains, the NHBPEP has an admirable record over the 15-year history of its existence. Results of a survey conducted in 1973 documented deficiencies in the general public knowledge regarding high blood pressure. This survey showed that the public not only lacked information regarding high blood pressure and its control, but was misinformed as well. For example, in 1973, many people confused hypertension with nervous tension. Although high blood pressure was a disease with a high prevalence, many Americans were unaware of this "silent killer" and consequently were not accustomed to having their blood pressure checked. The NHBPEP tried not only to inform the American public about high blood pressure and the consequences of its not being

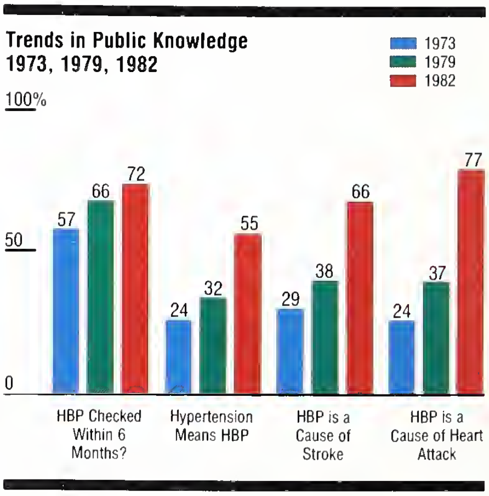


Figure 5. *(right)*
Between 1973 and 1982, the public's understanding of what hypertension means and its role in stroke and heart attack increased dramatically.

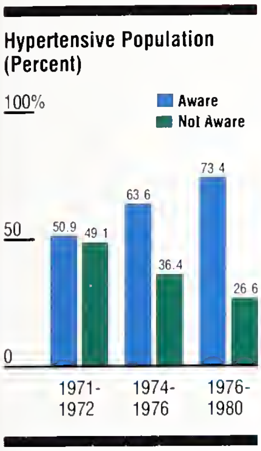


Figure 6.

Awareness of hypertension in Americans with hypertension increased between 1971 and 1980.

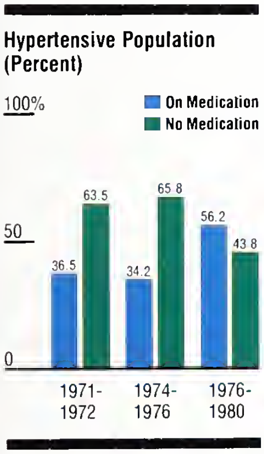


Figure 7.

More than 50 percent of Americans with hypertension were receiving medication for their disease by 1980.

treated, but also attempted to motivate the public to change its health behaviors. An aggressive campaign was mounted to inform Americans about these issues through printed materials and other media messages as well as to encourage them to visit their physicians to have their blood pressure checked. Figure 5 shows that, within a decade, there were marked improvements in the trends of public knowledge about high blood pressure.

Specifically, 72 percent of Americans reported having had their blood pressures checked within the previous six months. Understanding that hypertension is synonymous with high blood pressure increased from 24 percent of the public in 1973 to 55 percent in 1982. Public knowledge that high blood pressure is a cause of stroke also increased dramatically during this period, from 29 percent in 1973 to 66 percent in 1982. Similarly, understanding that high blood pressure can play a role in causing heart attacks increased from 24 percent to 77 percent by 1982.

The NHBPEP was established originally in 1972, in part because of the lack of awareness on the part of hypertensive patients of their disease. National Health and Nutrition Examination Surveys, conducted between the years 1971 and 1980, showed that the 50.9 percent of hypertensives who were aware of their disease in 1971 increased to 73.4 percent in 1980 (Figure 6).

Besides informing the public about the consequences of untreated high blood pressure and encouraging them to see their physicians, the NHBPEP also encourages patients with high blood pressure to stay on their medications. Figure 7 shows that in 1971 and 1972, a little more than one-third of the hypertensive population was on medication. By the period 1976 to 1980, that figure had increased to 56.2 percent. Lastly, perhaps the most encouraging information to emerge since the implementation of the NHBPEP has been the dramatic decline in age-adjusted death rates for stroke and coronary heart disease in the United States (Figure 2). Between 1968 and 1972, the stroke mortality rate declined by approximately 1.5 percent per year. Since 1972, the year in which NHBPEP was established, the decline in stroke mortality rate has accelerated to almost 5 percent per year. Over the past 15 years, stroke mortality has decreased by over 50 percent.

It is likely that there are multiple explanations for the overall decline in cardiovascular mortality, especially that due to stroke. In view of the steepness of the decline following the implementation of the NHBPEP, however, it is not unreasonable to assume that the aggressive advocacy for the control of blood pressure in the population by the NHBPEP has probably played a significant role in this impressive reduction of mortality.

Conclusion

High blood pressure is a disease with multiple and complex causes. It has a high prevalence, but there is great potential for prevention of complications through the control of blood pressure. A comprehensive national research effort has led to important new insights, but continued efforts in basic, clinical, and population-based investigations need to be sustained in order to meet the many challenges presented by this disease. As these challenges are met, the NHBPEP will continue to play a key role in the application of the products of research to the clinical setting.

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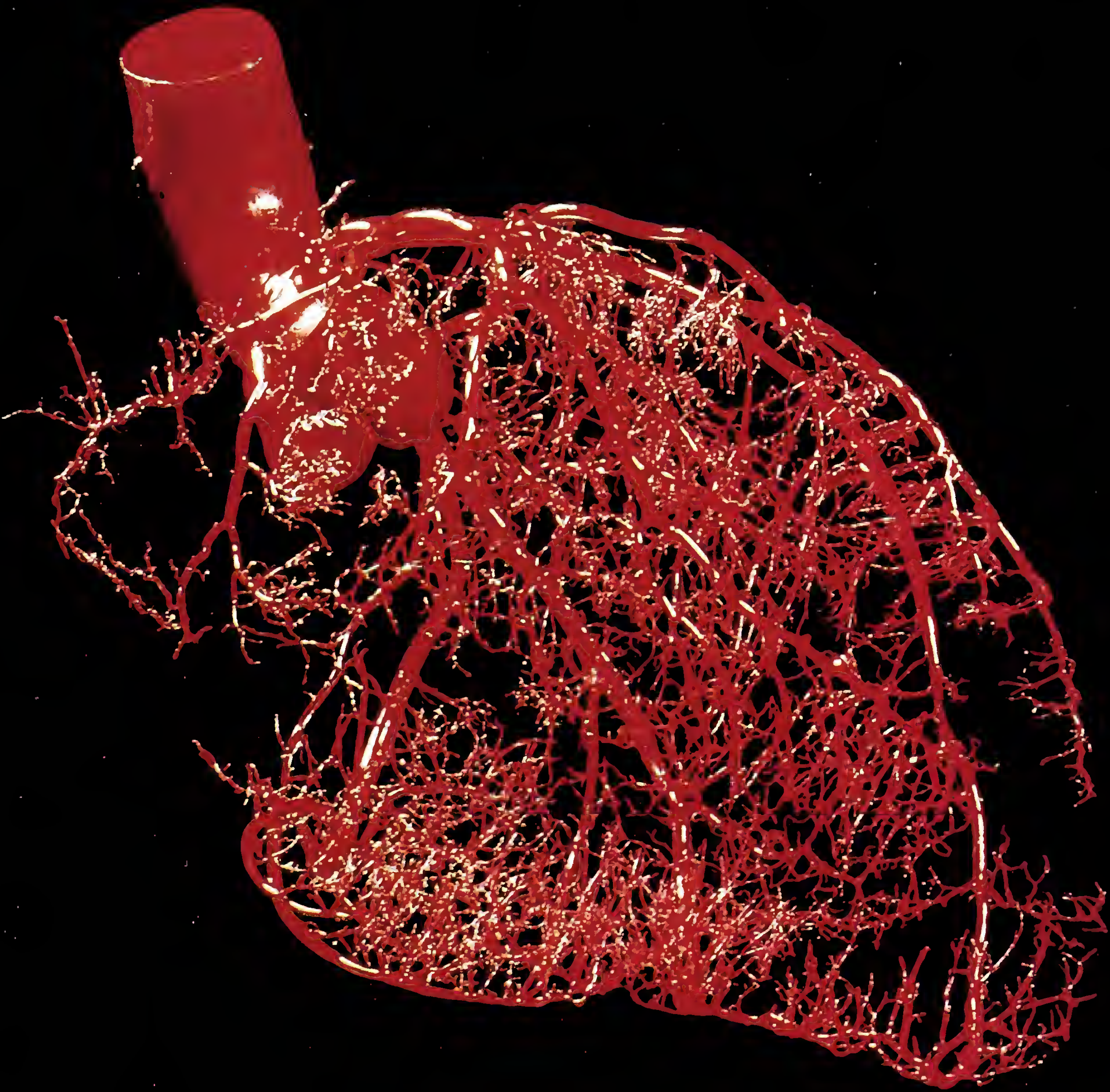
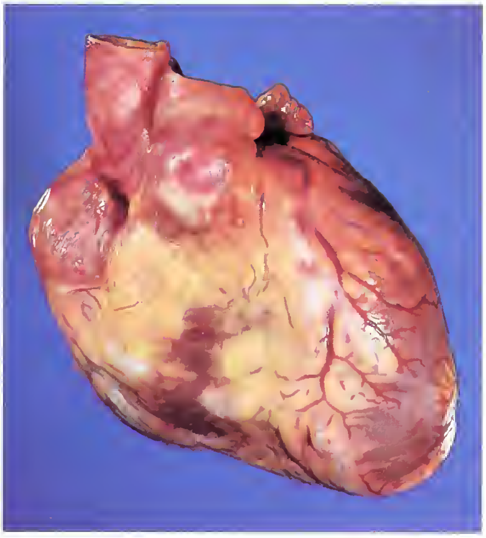


Heart Attack: Changing Concepts

The symptoms of a typical heart attack are well-known. Pain develops in the chest under the breast bone and radiates into the left shoulder and down the arm. It is accompanied by nausea, vomiting, and a cold sweat. Breathing may become difficult. The crushing sensation in the chest causes apprehension. What is not appreciated by many people is the dramatic change that has taken place over the past 40 years in our understanding of heart attack and in the effectiveness of its treatment.

(Left) The left coronary arterial system of the heart (a plastic casting).

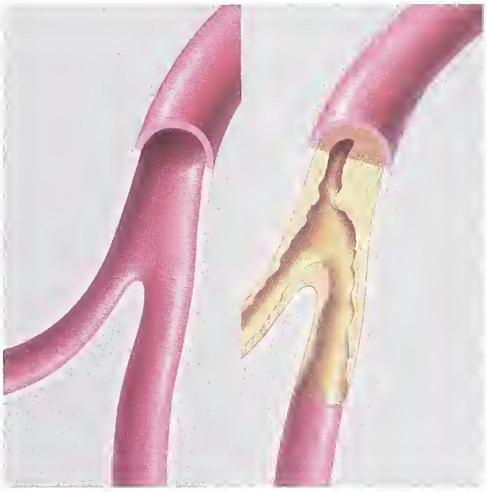


(Right) A normal heart (front view). The left anterior descending coronary artery and many of its branches are visible on the surface.

Sequence of Events

What happens when a heart attack occurs? A heart attack is a sequence of events leading to the irreversible damage of some heart muscle. The heart beats some 100,000 times a day; it literally does not get a second of rest. Thus, the heart muscle—the myocardium—requires an uninterrupted supply of blood through its arterial system to keep it supplied with oxygen and nutrients and to carry away carbon dioxide and the other waste products. The amount of blood needed by the heart depends upon its work load. The amount needed increases in response to physical exercise and even to emotional stress. The heart beats faster, pumps more blood with each beat, and ejects it at higher pressure. The blood supply to the heart consists of three major coronary arteries—the right, the left anterior descending, and the left circumflex coronary arteries—and their progressively smaller branches. The larger arteries are on the outer surface of the heart; their smaller branches go into the heart muscle and distribute themselves throughout the heart muscle.

The inner surface and inner layers of arteries may be thickened and otherwise changed in a process called atherosclerosis. The result is narrowing and hardening (arteriosclerosis) with a decrease in the amount of blood that can flow through. When the coronary arteries are involved, the result is coronary or ischemic heart disease, the most common form of heart disorder.



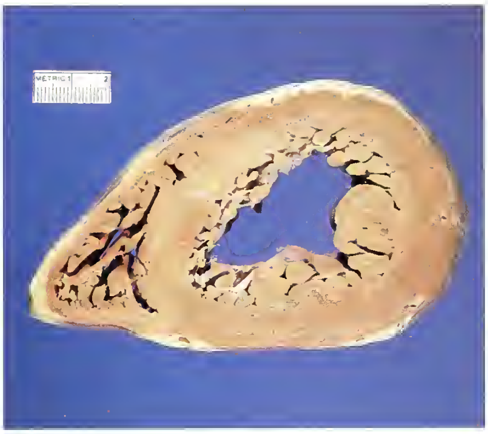
An illustration of normal and narrowed arteries.

When a coronary artery is seriously narrowed, the amount of blood that can flow to the heart muscle may not meet its needs when there is an extra work load, as during exercise. Exertion typically will result in a squeezing pain in the chest that may radiate into the left arm and elsewhere, but that disappears shortly after exercise stops. Such an episode is a temporary inadequacy of blood flow—or ischemia—and the pain that it produces is called angina pectoris.

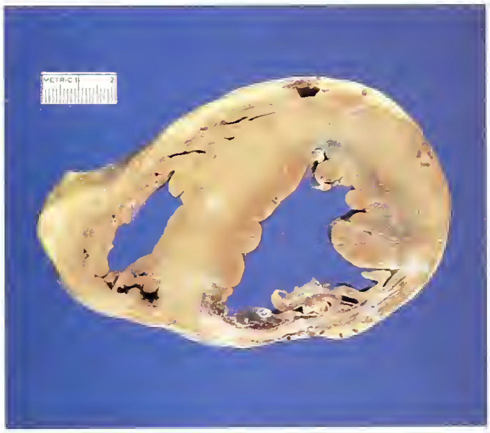
A narrowed coronary artery may occlude completely, typically because of a blood clot. When this happens, the downstream heart muscle fails to get enough blood to survive under its normal work load and, in a few hours, it undergoes irreversible damage and eventual replacement by scar tissue. This is a heart attack, or myocardial infarction.

Understanding the Cause

Scar tissue from previous heart attacks does not contract, and even seriously ischemic heart muscle loses some of its effectiveness in contraction. Thus, the heart becomes less effective as a pump. As the heart pumps less effectively, circulation of blood throughout the body may be reduced and blood pressure may fall. The brain and kidneys may not function well with decreased blood flow and may undergo irreversible damage, or the blood may "back up" in the lung and cause congestion and difficulty in breathing. Pumping impairment and its effects may range from completely insignificant to very severe, leading to shock and death. Even small heart attacks or ischemia without heart muscle damage can cause death because of disturbances in heart rhythm. Irregular rhythms may be set off; these may degenerate into a chaotic and ineffectual quivering of the heart muscle—ventricular fibrillation—that results in the cessation of the heart's pumping effectiveness and almost immediate death. The normal conduction of the electrical signal to the main pumping chambers of the heart also can be interrupted, resulting in a very slow or absent heart beat and, thus, another cause for inadequate pumping. Other problems can occur after a heart attack resulting in life-threatening situations. One of the heart valves may not close properly because the muscle that supports its restraining chords is damaged, and the heart muscle may tear. A clot may form inside the heart, break off, and embolize, which means that it is carried in the blood into successively smaller arteries until it can go no further. There, it obstructs blood flow and thus causes damage, especially if it obstructs blood to the brain. Or, with poor circulation, a clot may form in a large vein and embolize to the lung. The predominant problems with heart attack, however, are impaired pumping function and precipitously fatal rhythm disturbances.



Cross section of a normal heart. The postmortem fixed specimen is more brown and the chambers are smaller than in life. The larger chamber with the thicker walls is the left ventricle; the right ventricle is at the left of the illustration.



Cross section of a heart with an old myocardial infarction (a past heart attack). White scar tissue replaces muscle along the posterior surface.

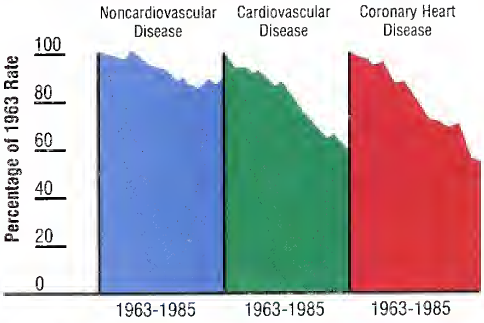
The interruption of blood flow in the coronary arteries on the surface of the heart is the result of two processes. One is the narrowing that develops gradually over years; it is due to atherosclerosis, and the resulting hardened and narrowed artery is said to be arteriosclerotic. The other is a relatively sudden obstruction at a previously narrowed site that is usually caused by a clot formation, which may be at the site of a tear in the inside wall of the arteriosclerotic artery. There is more recent evidence that, occasionally, a coronary artery may squeeze down, with the occlusion being the result of spasm.

Reducing Mortality From Heart Attack

Heart attack and coronary heart disease have been the leading cause of death in the United States since about 1940. This public health problem continued to increase until the middle 1960s when coronary heart disease accounted for one in three deaths. In the past two decades, this trend has been reversed dramatically, and these deaths now constitute only 26 percent of the total. The average age of the American population has increased during this period and, when this is taken into account, the age-adjusted mortality rate actually has decreased by 42 percent. Despite this progress, the problem is far from solved. Coronary heart disease is still the leading cause of death and a leading cause of discomfort and disability.

The recent decrease in coronary heart disease mortality is attributed to two factors—a decrease in the occurrence of coronary heart disease, and more effective ways of treating it. The decrease in occurrence of the disease seems to be a result of improvements in our lifestyles. A smaller segment of the population is smoking, high blood

Twenty-two Year Trends in Age-Adjusted Death Rates, U.S.A., 1963-1985



Source: NCHS

U.S. coronary heart disease, all cardiovascular disease, and noncardiovascular disease age-adjusted death rate trends, as a percentage of 1963 rates.

pressure is better controlled, Americans are eating less saturated fats, and blood cholesterol level in the population is decreasing. Physical activity is becoming more a part of our lifestyle. Health education programs have been initiated and changes in public behavior have taken place. Research also has brought about new understanding and more successful management of heart disease. Some of the changes in the management of acute myocardial infarction are discussed below in greater detail.

Progress in the Care of Heart Attack Patients

Forty years ago, myocardial infarction was diagnosed on the basis of clinical symptoms, signs of compromised heart pumping, and changes in the electrocardiogram. But symptoms may be mild or uncharacteristic, the physical examination may be normal, and the electrocardiogram may not show immediate changes. Few blood tests available forty years ago would show any change and even those changes, such as an increased sedimentation rate of the blood cells and increase in the number of white blood cells, could be caused by other problems. Additional confirmatory physical findings and changes in the electrocardiogram might evolve, but mild cases were often missed. Treatment consisted of relieving pain, giving oxygen, and imposing complete and prolonged rest.

Anticoagulant drugs were coming into use, with the goals of avoiding more clotting in the coronary arteries and preventing clotting elsewhere that might lead to embolization. A limited number of drugs were available for the treatment of such complications as heart failure, shock, and rhythm disturbances. The underlying concept of therapy was to allow the body to heal itself. Slow recovery with return to a substantially reduced level of activity over 6 or more months was considered a medical success. Of those admitted to the hospital, however, one-third died and many more died before they got to the hospital.

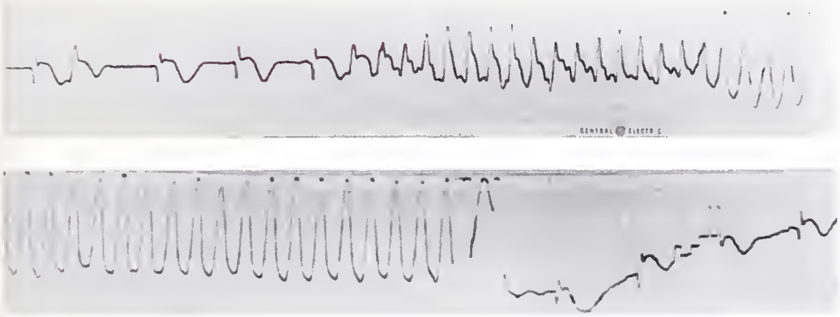
Today, those suspected of a heart attack are brought quickly to the hospital. Many new therapies exist, including those that reverse some incipient heart muscle damage. Of those hospitalized, 85 percent or more survive. Recovery is hastened, with many patients discharged within a week and back to their normal activities in 6 to 8 weeks.

What led to these dramatic changes in the management of heart attack? It is interesting to examine some of the major factors.

The rhythm of the heart is reflected in its electrical activity, which is one of the important features monitored in heart attack patients in the coronary care unit.

Developing Better Resuscitation Techniques

The first major developments were steps toward more effective resuscitation techniques and attempts to apply them to patients dying of a heart attack. In 1933, a method of cardiac resuscitation was developed in animals, but it required opening the chest, rhythmic massage of the heart, and then electrical reversal of ventricular fibrillation. Electrical reversal of ventricular fibrillation was first applied to a human in 1947, when this fatal rhythm disorder developed during an operation inside the chest and the heart was easily accessible. Three years later, two men, each of whom had developed seemingly fatal heart attacks in the hospital, were resuscitated by emergency opening of the chest, heart massage, and defibrillation.



An electrocardiographic rhythm strip showing the development of ventricular fibrillation (upper panel). This is terminated by electrical defibrillation (tracing off the chart in lower panel) which is followed by the development of normal heart rhythm.

Techniques for defibrillation and pacing through the intact chest wall were developed a few years later, but the need to open the chest and squeeze the heart for maintaining the circulation stood in the way of applying these techniques widely. In 1960, this problem was overcome with the demonstration that some circulation could be achieved, without opening the chest, by regular compression on the breastbone and chest. (It was long thought that this process represents massaging the heart, but it is actually a bellows action of the chest.) This procedure, combined with mouth-to-mouth respiration, allowed maintenance of the circulation until the disorder of the heart rhythm could be controlled by defibrillation. These developments led to the establishment of the coronary care unit (CCU) to serve as a facility in which heart attack patients could be observed so that attempts at resuscitation could be started promptly. Soon, their value became apparent as an environment that allowed the early recognition of cardiac problems and their effective treatment.

Managing Rhythm Disturbances

The existence of cardiac rhythm disturbances with heart attacks had long been recognized, but it was not until the early 1960s that their importance was appreciated. It was then noted that seemingly minor rhythm disturbances—ventricular premature contractions and other signs of ventricular irritability—were very common early during a heart attack; that ventricular fibrillation, the chaotic and ineffectual quivering of heart muscle, was the cause of about one-half of the heart attack deaths in the hospital; and that ventricular fibrillation generally was heralded by ventricular irritability that would be considered benign in other circumstances. These findings, and developments in engineering technology, led to an emphasis upon constant monitoring of the heart rhythm and the early administration of antiarrhythmic drugs to prevent the development of fatal rhythm disturbances. Lidocaine, a drug used as a local anesthetic, was found to have significant antiarrhythmic properties and it could be given intravenously to achieve prompt control. Thus, the prevention of fatal rhythm disturbances took the place of resuscitation as the primary goal of the coronary care unit.

But, almost two-thirds of heart attack deaths were occurring before patients were admitted to the hospital. When the importance of rhythm disturbances in sudden death was recognized and methods for managing these rhythm disturbances became available, emphasis was placed upon early hospitalization of patients with the suspicion of heart attack. Emergency care was also upgraded. By the late 1960s, some ambulances were being equipped with coronary care facilities. Ambulance personnel received special training, and radio links transmitted the electrocardiogram and provided voice communications with physicians. These developments and efforts for better care of trauma victims led to the major improvement in emergency care systems in this country in the 1970s.

The Role of Clinical Research

Despite such advances, the management of myocardial infarction was still largely pragmatic. Research was being focused on the underlying problem of atherosclerosis and, to a limited extent, on the rhythm complications of heart attack, but very little attention was being directed to understanding the full scope of heart attack or its possible therapy. Thus, in 1966, the then National Heart Institute undertook the development of a broadly based clinical and fundamental research program focused on myocardial infarction—unraveling the fundamental physiological disturbances, characterizing the course of the acute illness, and developing more effective therapies. A network of nine Myocardial Infarction Research Units was established; they were the forerunners of the Specialized Centers of Research that are now directed at several important clinical problems within the purview of the National Heart, Lung, and Blood Institute. The first major task of the program was the development of research coronary care units. In these facilities, patients could be monitored and treated at least as well as in the coronary care units, but more extensive physiological and clinical observations could be made. In immediate proximity to the units, special x-ray and cardiac catheterization facilities were made available.

New techniques were developed for characterizing the effects of impaired heart muscle and its pumping. One of the most important of these new techniques has permitted the measurement of blood pressure inside the right side of the heart and in the pulmonary vasculature without the need for moving the patient from the bed. A special catheter with a deflated balloon at its tip could be inserted into a forearm vein and advanced through the venous system to the right heart, where the balloon would be inflated; the distended balloon, and thus the catheter tip, would be carried into the right heart and on into the pulmonary arterial system. This Swan-Ganz catheter, which was developed as a research method, has evolved into a widely used clinical tool for assessing patients with heart attack and other circulatory problems. It also has been further refined so that other functions can be performed with the catheter, including sensing electrical signals within the heart, measuring blood flow, and pacing the heart.

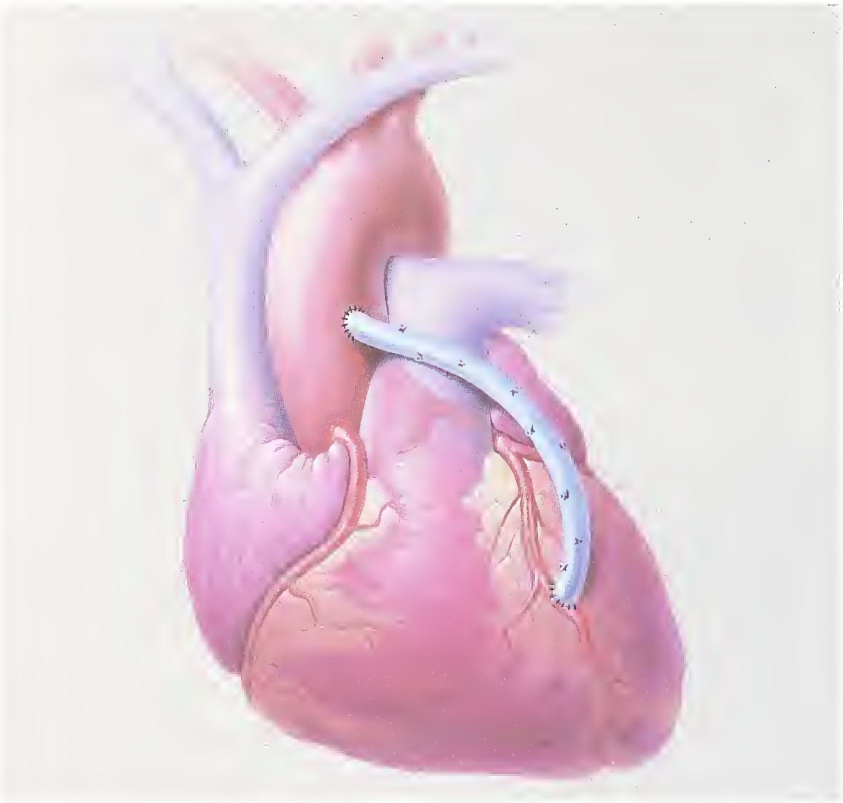
Research in this setting led to a much more extensive characterization of the complexity of heart attack and the responses of the body—the hemodynamic, endocrine, and metabolic effects, as well as common psychological and emotional consequences. New therapies were evaluated. The feasibility of extensive physiological studies was demonstrated, even in the face of acute illness. In addition to the important observations about myocardial infarction, it was found that patients were not nearly as fragile as had been thought and could undergo much more extensive investigation and activity than had been recognized previously.

A Swan-Ganz catheter. The contents of the syringe at one end have been used to distend the balloon at the other end.

Early in this research program, the thrust of efforts shifted from seeking to prevent death from heart attack to reducing the amount of damage caused by a heart attack. The underlying tenet was that the amount of heart muscle damage was not irreversibly determined at the time of admission to the hospital. Research in animals demonstrated that irreversible heart muscle damage occurred only after about a half-hour of abrupt, complete coronary artery occlusion; further research has shown that the window of opportunity for preventing or reducing heart muscle damage extends for several hours in the typical clinical setting. Three major lines of research were pursued: decreasing the work load upon the heart by a variety of manipulations; modifying the biochemical environment of heart muscle with the goal of protecting it; and reperfusing the occluded vessel, including the use of substances that would dissolve or "lyse" blood clots.

Adapting Other Cardiology Techniques

Some of the major developments in the treatment of acute myocardial infarction came from progress on other problems in cardiology, particularly the management of chronic coronary heart disease. For example, a new x-ray technique, coronary angiography, permitted visualization of coronary arteries and their narrowings. A catheter is threaded into an artery in the arm or groin, back to the heart, and into the orifice of one of the coronary arteries for the injection of a liquid that absorbs x-rays. In the late 1960s, soon after the anatomic details of coronary artery disease could be characterized by this x-ray technique, a surgical procedure was developed to overcome the



An illustration of the result of a coronary artery bypass procedure. A vessel is positioned to allow blood flow from the aorta (the large artery leading from the heart) to the left anterior descending coronary artery, downstream from a narrowing.

effects of coronary obstruction. In coronary artery bypass surgery, a vein from a leg or an artery on the inner chest wall is transplanted to allow blood flow around the site of coronary obstruction.

About a decade later, a method was developed to relieve the obstruction itself by a catheter technique, thus avoiding the need for surgery in some patients. In this percutaneous transluminal coronary angioplasty procedure, a catheter with a tiny deflated balloon at its tip is advanced through the arterial system so that the balloon is at the site of coronary obstruction; the balloon is briefly inflated at high pressure to dilate the narrowing and relieve the coronary obstruction. Drugs used in the management of angina pectoris, such as the beta-blockers that diminish the work load in the heart and, more recently, the calcium channel blockers, are used in acute myocardial infarction. Developments in the management of chronic congestive heart failure, such as the use of vasodilator drugs, also have been applied to acutely ill patients.

Advances in Diagnosis and Evaluation

Many diagnostic and evaluative techniques have been utilized. For example, enzymes released by damaged heart muscle can be detected in the blood and used to help diagnose a heart attack early or to recognize the progression of heart attack. The first of these enzymes, found by a medical student working in a research laboratory one summer, was quite useful. It is, however, released by injured or congested liver, skeletal muscle, and other sources. Further research identified an enzyme (the MB isoenzyme of creatine kinase), that is almost unique to heart muscle and is therefore specific for the diagnosis of myocardial infarction when detected in the bloodstream. A number of radioisotope techniques are very useful; some radioisotopes localize in damaged heart muscle and others identify healthy heart muscle.

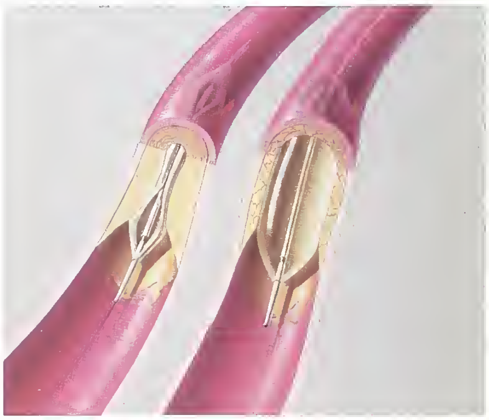
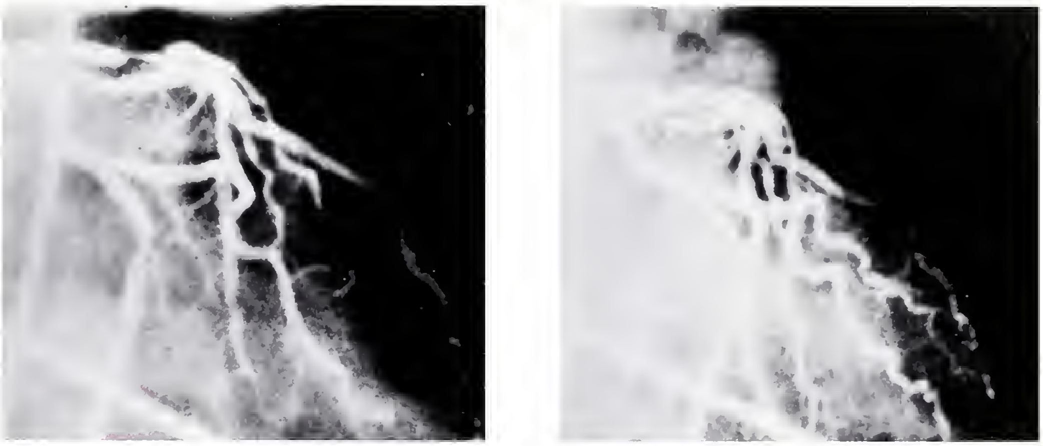


Illustration of the percutaneous transluminal coronary angioplasty procedure. At left, the catheter-tip balloon has been positioned at the site of arterial narrowing. At the right, the catheter is distended to remold the artery and relieve the narrowing.



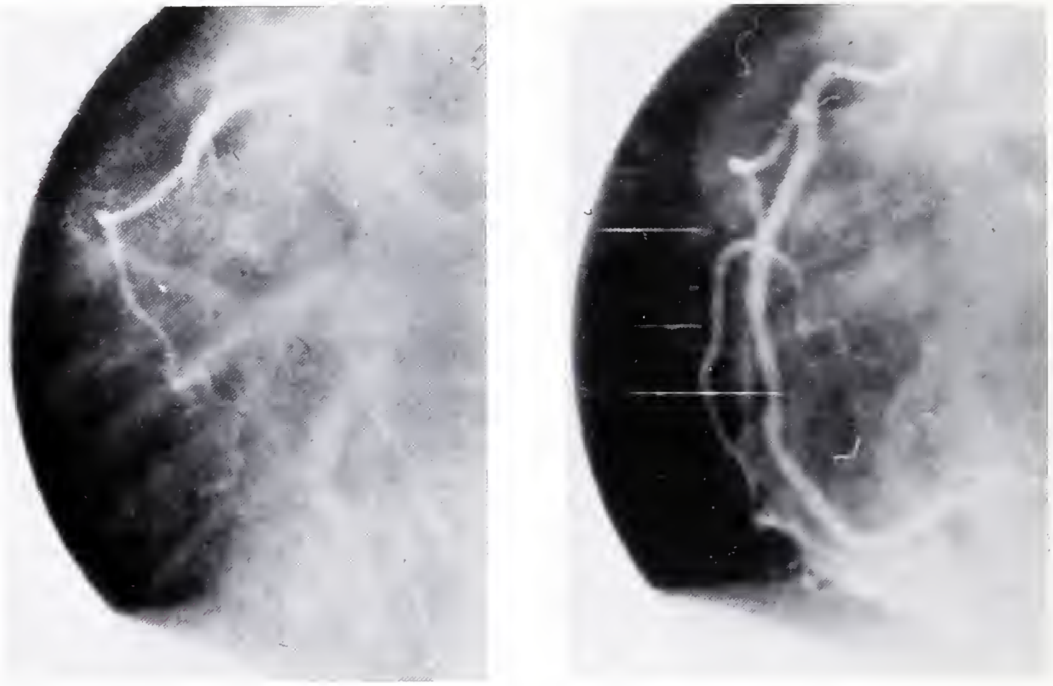
X-rays of the left coronary artery system (with injections of X-ray opaque dye in it) before and after percutaneous transluminal coronary angioplasty (PTCA). The coronary angiogram on the left (before PTCA) shows a serious narrowing in the artery at the top center which is opened up after PTCA, as shown in the angiogram on the right.

Still other radioisotopes that are tagged onto substances that stay in the blood can be used to characterize the pumping performance of the heart. Ultrasound techniques—echocardiography—also can estimate pumping effectiveness and can detect such structural abnormalities as damaged heart valves. The electrical activity underlying rhythm disturbances can be characterized more precisely by special signal sensing and processing systems. Such x-ray techniques as ventriculography and coronary angiography can visualize the main pumping chambers and the coronary arteries with great precision. New imaging techniques, such as magnetic resonance imaging and positron emission tomography, are being used to characterize metabolic function and to provide images of heart structure. A radioisotope-tagged antibody to a substance in clotted blood attaches to a blood clot and can be used to identify these occluded lesions in a coronary artery. This diagnostic process is being modified into a therapeutic technique to carry drugs more effectively and directly to occlusions.

Current Concepts in Management

Today, the concepts and management of myocardial infarction are vastly different from what they were 40 years ago. Early recognition and very early treatment are at a premium because a major goal is to avoid or at least to reduce the amount of heart damage. Rhythm disturbances can be detected more reliably and controlled with greater effectiveness. The changes in the circulatory system that are associated with myocardial infarction are better understood, and therapy can minimize the work load on the heart yet maintain an adequate circulation.

Most dramatically, blood clots in the coronary arteries are being attacked directly. Substances that dissolve fresh blood clots—thrombolytic agents—are being utilized. When the thrombolytic substances initially available were tested with intravenous use, the results were not persuasive. Subsequently, thrombolytic substances were introduced directly into the affected coronary artery by cardiac catheterization. This procedure produced effects that were often dramatic and directly visible on the angiogram, but the technique

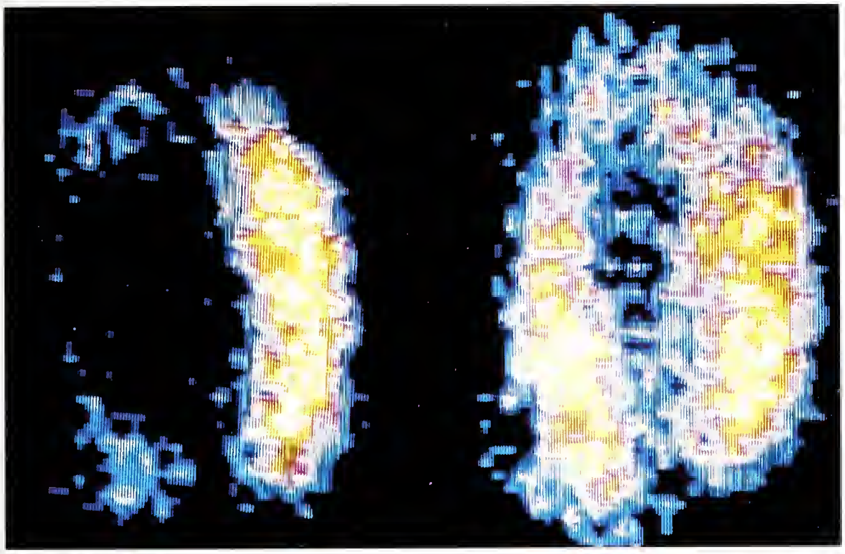


A right coronary artery before (left) and after (right) dissolution of a clot by a thrombolytic agent. Note the extensive arterial system that did not fill with dye because of the occluding clot; it fills readily after thrombolysis.

required special facilities and almost immediate cardiac catheterization to permit administration of the drug within 4 hours of the onset of symptoms. Even when facilities are available, delays are unavoidable, and intracoronary drug administration is often too late. But, the beneficial effects of intracoronary thrombolysis prompted new looks at early intravenous administration of existing agents and the identification, purification, and synthesis of other thrombolytic agents. With the use of molecular biology and biotechnology, thrombolytic substances that exist in the human body have been reproduced and synthesized in large quantities. These agents seem even more effective and more free of side effects. Thrombolytic agents have now been shown to be effective in the majority of patients if they are given early after the onset of myocardial infarction, even when given intravenously. Thus, the therapy can be used widely, quickly, and without special facilities.

Although early thrombolytic therapy breaks up perhaps three-quarters of the occlusive clots, the underlying chronic coronary narrowing remains, and the likelihood of recurrent obstruction is significant. Current techniques permit relief of the obstruction with percutaneous transluminal coronary angioplasty even while the heart attack is fresh; whether this therapy is beneficial during the immediate hours after a heart attack or later during hospitalization is yet to be determined.

The fundamental concept today is to minimize damage and to assure a prompt recovery, swift rehabilitation, and return to what is often a more active lifestyle than existed before the heart attack. In many hospitals, a patient with uncomplicated heart attack is discharged in 7 to 10 days, a marked contrast from the 4 to 6 or more weeks that was common in the past. Active rehabilitation is practiced. Even before the patient leaves the hospital, cardiac function is often assessed by using carefully controlled exercise testing to help guide the rehabilitation. Major attention is given to lifestyle factors to reduce risk—modifications in diet, exercise habits, weight, and smoking avoidance.



Radioisotopic images of a heart during ischemia (left) and after ischemia (right). The two pictures are like vertical cross sections of the heart, with yellow-orange-red-violet representing progressively lesser concentrations of radioisotope. An area of ischemia—the left half of the left image—is reflected by an absent uptake of the injected isotope.

Future Directions

Major challenges remain in the management of acute myocardial infarction. Further reductions in the development of the underlying atherosclerotic disease are of fundamental importance. The factors that convert chronic coronary heart disease to acute myocardial infarction must be clarified so that strategies can be developed to interrupt or avoid them. In the treatment of myocardial infarction, optimizing thrombolytic therapy, the choice of thrombolytic agent, and a clarification of the role of mechanical relief remain important issues. More effective management of heart failure when heart muscle damage is extensive remains a challenge. The momentum of advance continues.

Peter L. Frommer, M.D.

FRAMINGHAM HEART STUDY
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Risk Factors for Cardiovascular Disease: The Framingham Heart Study

Framingham participants have their periodic checkups in a white frame house that serves as the study's medical facility.

The Framingham Heart Study began over 40 years ago almost coincident with the beginning of the National Heart, Lung, and Blood Institute. Dr. Joseph Mountin, an Assistant Surgeon General of the United States Public Health Service, proposed to use the methods of a science known as epidemiology to study why people get heart attacks, strokes, and other degenerative vascular diseases related to atherosclerosis or "hardening of the arteries". Up to that point, epidemiology, which means literally the study of an epidemic, was used to study infectious diseases and nutritional epidemics. Doctors and health scientists would go into a town that had an epidemic of typhoid fever and locate the sources of the germs and how they were transmitted, and then use all the information to stop the epidemic. Dr. Mountin reasoned that perhaps these same techniques could be used against the number one epidemic afflicting the country, which was coronary heart disease. He felt that if enough was learned about how this disease developed in a free-living population, enough clues might also be found that would lead us to the best way to prevent this disease. Although many experts in epidemiology of that day insisted that epidemiology could not be used to study a chronic illness, he persisted in his plan to find a town in the United States to do this project.

The Early Years

A number of locations had been suggested as a site for the study. Instrumental in the selection of Framingham was Dr. David D. Rutstein, who had just arrived in Boston to become Chairman of the Department of Preventive Medicine at the Harvard Medical School. After looking at possible sites in Massachusetts, the town of Framingham was selected for the study because it had been used in the years 1917 through 1923 by the Metropolitan Life Insurance Company to study tuberculosis in a free-living population. The Metropolitan Life Insurance Company sent 70 doctors and nurses into Framingham to take sputum and other cultures to identify how tuberculosis is spread and to understand how to curtail this problem. Because the town already had experience with medical researchers, it was decided to use it for the study of heart diseases. In many respects, Framingham turned out to be an ideal choice. It was far enough away from Boston so that the people used the local facilities for health care. This made follow-up much easier. Moreover, it was close enough to Boston so that the study doctors could obtain the expertise of many specialists in Boston. Through the years, these specialists have continued to come to Framingham to give advice. By October of 1947, a team under the direction of Dr. Gilcin Meadors had arrived in Framingham to organize the study. It was July 1, 1949, when Dr. C.J. Van Slyke, the first director of what was to become the National Heart, Lung, and Blood Institute, assumed responsibility for what was then called the Framingham Heart Disease Epidemiology Study.

After the study came under the Institute's purview, a new protocol was developed, largely by Mr. Felix Moore. It was implemented by Dr. Thomas Royle Dawber who directed the completion of the first cycle of the study of the population. His leadership provided for most of the techniques and policies that governed the operation of the Framingham Heart Study.

From its beginning, the subjects of the Study, who represent every other man and woman in the town of Framingham between the ages of 30 and 62 years—in all, some 5,209 men and women out of the total population of 10,000 adults in Framingham—have been examined every two years to determine their health status and particularly to identify those who develop the sequelae of the atherosclerotic process. Many other diseases have been studied at Framingham as well, including lung diseases, cancer, and eye diseases.

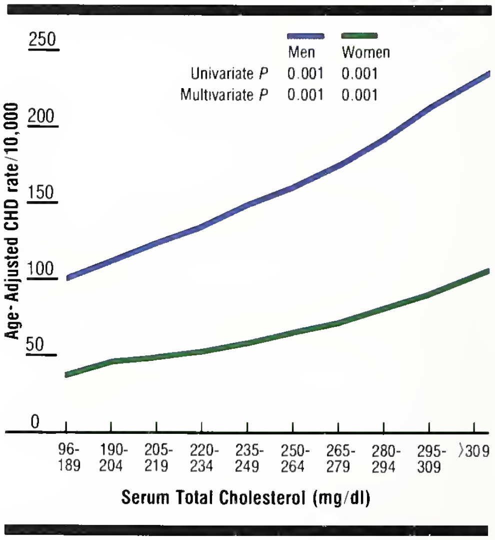
One of the first findings of the Framingham Heart Study was the rate of heart attack that afflicted the population. In the first 14 years of the study, every eighth man, 40 to 44 years old, developed some form of coronary heart disease (CHD). Every sixth man, 45 to 49 years old; every fifth man, 50 to 54 years old; and every fourth man, 55 years or older, also developed some clinical manifestation of this disease, ranging from angina pectoris (perhaps its mildest form) to sudden death (its most devastating form). By the age of 60 years, every fifth man in the town had become a victim of CHD in one form or another. Coronary heart disease prevalence rates are similar throughout the United States. Before menopause, Framingham women fared better than men, but after menopause, their CHD rates caught up with those of men of the same age.

The Concept of Risk Factors

The way things were done in Framingham went something like this. People entered the study in good health and many things were measured that are traditionally measured by doctors, such as blood pressure, blood sugar, and cholesterol. Other measurements were taken, however, that relate to life style and life habits that were not usually considered in those days. The study

Figure 1.

Average annual incidence of coronary heart disease in men and women aged 45 to 74.



participants were then followed every two years by periodic medical examinations. Some went on to develop heart attacks; some did not. It became possible, thereby, to compare the people who did get a heart attack with the people who did not on the basis of the many things that were measured in each group when the study participants were healthy many years before.

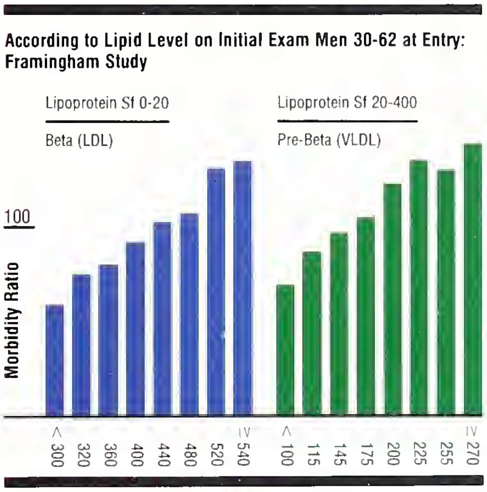


Figure 2.

Coronary heart disease morbidity and lipid level at entry into study in men aged 30 to 62.

Anything that you could measure that became associated with a higher rate of heart attack or stroke later in life became known as a risk factor.

Framingham was one of the first studies to develop the concept known as the risk factor. A risk factor may play a role in producing disease. To establish such a connection, however, intervention trials must be done. And so, it has been, over the years, that someone would look at the evidence in Framingham, for example, of the role of cholesterol in producing heart attacks and design a study to lower cholesterol to see if it was possible to prevent a heart attack. Indeed, it is of no little consequence that if you lower blood cholesterol levels, you lower the rate of heart attack—but we are getting ahead of the cholesterol story which follows.

Cholesterol and Heart Disease

Blood cholesterol level was one of the first major risk factors for CHD identified in Framingham. Data from the first 18 years of the study show the association of blood cholesterol level with the incidence of CHD (Figure 1). For every 1 percent increase in blood cholesterol level, a person's chance of heart attack increased by 2 percent. During the first 30 years of the Framingham Study, more than 90 percent of people with blood cholesterol levels over 300 mg/dl developed a heart attack. The average blood cholesterol level in men who had a heart attack was 244 mg/dl; however, most heart attacks occurred in men whose blood cholesterol levels were between 200 and 270 mg/dl.

The cholesterol story is very complex. Cholesterol is carried in our blood in association with five classes of fat packets, or particles, that we call lipoproteins. Of these five, at least three have been identified as playing major roles in the development of CHD: very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). As Figure 2 shows, LDL levels are related in linear fashion to CHD morbidity. People with the highest levels of LDL-cholesterol have a genetic disease, familial hypercholesterolemia (FH). For their work on this subject, Drs. Michael S. Brown and Joseph L. Goldstein received the 1985 Nobel Prize in Physiology or Medicine. Some people with FH develop CHD at a very young age. Also shown in Figure 2 are data from the Framingham Study on VLDL which transports most of the triglyceride in blood. As can be seen, the higher the blood triglyceride level, the higher the heart attack rate. The third major class of cholesterol-transporting lipoprotein is HDL which, in contrast to LDL and VLDL, can have a protective effect with respect to CHD.

Statistical Analysis and Risk Factors

Framingham pioneered progress in the use of statistical analysis to determine how risk factors are associated with disease. In any disease, there are usually many factors involved, some of which will be associated with other factors that may not contribute to the actual cause of the disease. To understand these complex relationships and to get at the most direct causes of any disease, multivariate or multilogistical statistical tests are performed. These tests try to determine what part of an association between a factor and a disease is due to the factor directly and not to its association with other factors that are also related to that disease. The statistical methodology developed during the Framingham Study allows us to look at the independent contribution of a factor to disease risk. It allows us, for example, to isolate the role of triglyceride for close examination.

Blood triglyceride level was found to be an independent risk factor for CHD for women, but not for men, as shown by multivariate analysis; thus, it has been the object of much debate. Lately, it has been shown that people with high blood triglyceride levels and low levels of high-density lipoproteins are at risk of heart attack as well as diabetes mellitus. This group is also likely to have high uric acid levels that could lead to additional health impairment. These findings appear to apply equally to men and women.

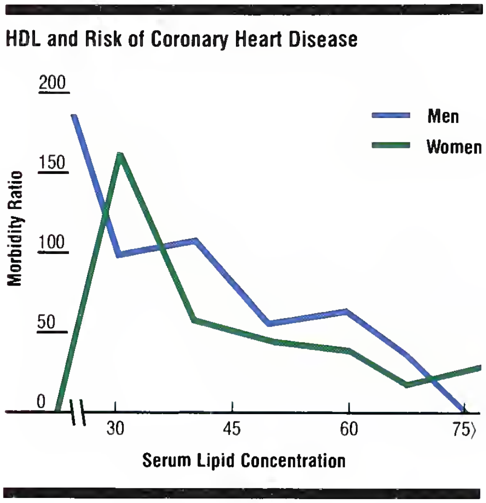


Figure 3.

Incidence of coronary heart disease by high-density lipoprotein cholesterol level in men and women aged 50 to 80 years.

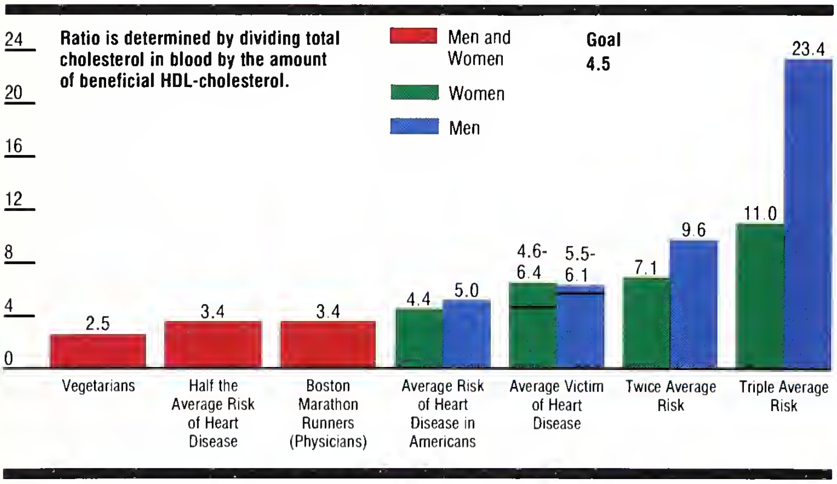
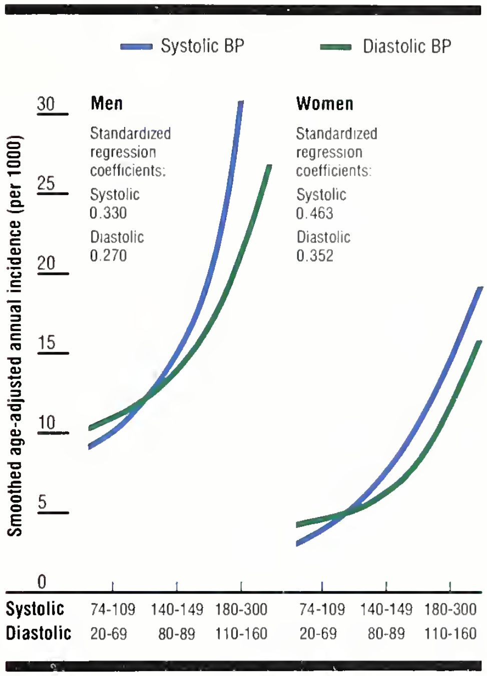


Figure 4.

Cholesterol ratio (total blood cholesterol: HDL-cholesterol) as a clue to risk of coronary heart disease.

HDL-Cholesterol—The "Good" Cholesterol

Another element of the cholesterol-heart attack relationship is the role of HDL; the higher the HDL blood level, the lower the subsequent risk of CHD (Figure 3). This finding prompted the *Reader's Digest* to call HDL-cholesterol the "good kind of cholesterol." High-density lipoprotein particles initiate the transport of cholesterol from arterial wall deposits back to the liver, from which 95 percent of the daily cholesterol excreted leaves our bodies (through the bile duct and intestines). The higher the HDL level in blood, the lower will be the total amount of cholesterol in the body.



*Framingham study, 20-yr follow-up

Figure 5.

Incidence of coronary heart disease by blood pressure level in men and women aged 45 to 74 years.

A simple way of determining a person's lipid profile is to measure just two kinds of cholesterol in the blood stream—the total cholesterol and the HDL-cholesterol—and divide the smaller number (the HDL part) into the larger number (the total cholesterol part). As Figure 4 shows, the higher this ratio, the higher the risk of heart disease. We would recommend that all Americans try to achieve a ratio below 4.5, not because 4.5 is absolutely safe—3.5 is closer to a safe number—but primarily because 4.5 is a signal that tells a person to adjust his or her lipid profile. One-half of all Americans die from atherosclerotic disease and the sooner they start to correct their blood cholesterol levels, by diet and exercise especially, the better it will be for them.

Blood Pressure

A different but very important risk factor found in Framingham was the relationship of blood pressure to atherosclerotic disease and also to stroke. Figure 5 shows the striking association of blood pressure (BP), whether systolic or diastolic, to CHD incidence. Compared to people with blood pressure levels in the normal range, if you have high blood pressure (a systolic BP of 160 mm Hg or a diastolic BP of 95 mm Hg or higher), you can expect to run twice the risk of heart attack, seven times the risk of stroke, and two to three times the risk of other cardiovascular disease, such as congestive heart failure or peripheral vascular disease. Even borderline high blood pressures (systolic BP of 140 to 159 mm Hg, diastolic BP of 90 to 94 mm Hg) are not healthy. They have been shown to produce a 50 percent increase in the heart attack rate (three times the rate of stroke), and substantial increases in the rates of some of the other vascular diseases. Hypertension intervention studies conducted since the 1960s in this country have shown that lowering blood pressure can reverse some of these diseases, especially stroke.

Cigarette Smoking

The third major risk factor identified in Framingham was cigarette smoking. Smoking cigarettes, filtered or not, doubles the rate of heart attack. Men under age 55 who switched to filter cigarettes, as shown in Figure 6, did not reduce their risk during the past 15 years in Framingham. People

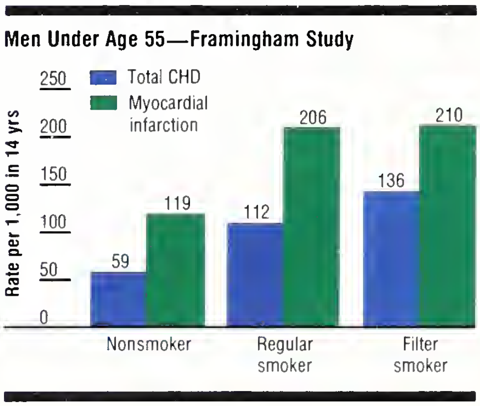
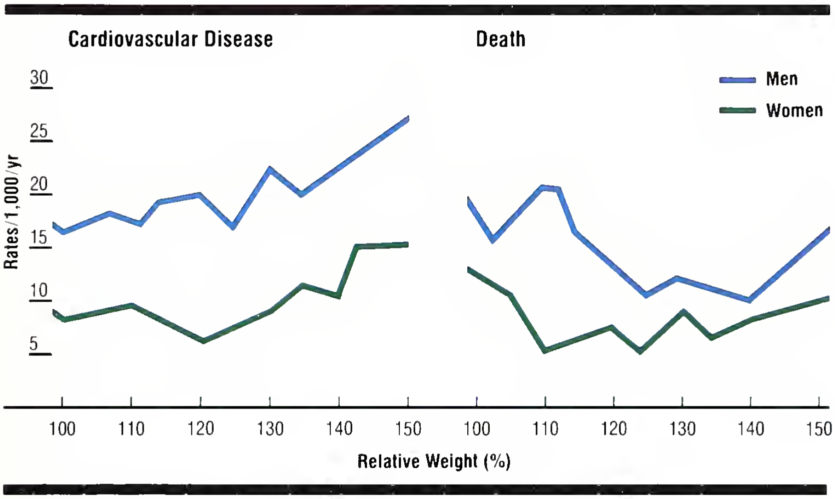


Figure 6.

Cigarette smoking and risk of coronary heart disease in men under age 55.

who stopped smoking, however, reduced their risk of heart attack so that, by a little over a year after cessation, their risk of heart attack was equivalent to that of people who had never smoked. Cigarette smoking also curtails lung function resulting in diseases such as chronic bronchitis (which usually results in bouts of pneumonia later in life), emphysema, and, all too frequently, lung cancer. For just about every cigarette a person smoked a day in the first 30 years in Framingham, his or her lung cancer rate rose one time. Two cigarettes a day doubled the rate, three tripled it, and a pack a day increased the lung cancer rate by almost 20 times.



*Framingham study 18-year follow-up; data shown are crude rates for the age group 45-74.

Figure 7.

Five-year incidence of cardiovascular disease and death by relative weight.

Obesity

Obesity is a major risk factor. As you ascend the weight scale in Framingham, your subsequent rate of cardiovascular disease rises, as seen on the left side of Figure 7. The greater the weight, the higher the rate of heart attack and stroke. As simple as this relationship seems, however, there were doubts raised about this finding. For example, we learned very early in the Framingham Study that as people gained weight, they also changed many of their standard risk factors. Their LDL and VLDL (triglyceride) cholesterols would rise; HDL cholesterols (the good cholesterols) would fall. Their blood pressures would rise, and their blood sugars and uric acids also rose. In other words, a host of risk factors got worse as weight increased. Indeed, when we did some very detailed statistical analyses to determine how much of the change in the rate of cardiovascular disease was explained by the change in these traditional risk factors, the early results suggested that practically all the risk could be explained, not by the obesity itself, but by the concomitant changes in the risk factors. This suggested that if you got fat without changing any risk factors, while you would still pay a price in your weight-bearing joints and a psychological price for not being thin and beautiful, you would not pay a price in terms of cardiovascular disease. As follow-up data accumulated, however, we learned that if you follow the people who gained weight but who did not change the other risk factors, they eventually develop higher rates of cardiovascular disease. It took eight years to see this in the men and fourteen years to see it in the women.

Obesity is now considered an independent risk factor but even when it was not, it had to be one of the most important risk factors because it changed so many other risk factors that are not healthy. Unfortunately, the obesity story did not end there. Other people started to look at the effect of obesity on death from all causes and saw a U-shape curve, similar to the curve on the right hand side of Figure 7, and they all said "my goodness, don't be skinny, those people have a very high death rate!" We at Framingham became interested in this curve on death and soon learned that the people who die fat, die of heart attacks and strokes, and the people who die thin die of cancer. And so we looked at death in smokers and non-smokers and, as you can see in Figure 8, if you are a thin non-smoker, you will have the lowest death rate in the next 30 years. Non-smokers who gain more than 10 percent over the 1959 Metropolitan Life Insurance Company Weight Scales die at four times the rate of people who are at ideal weight for their inches of height on those scales. People who are 20 percent overweight die at almost 4.5 to 5 times the rate of thin non-smokers. However, if you smoke and are thin, you die at nine times the rate of the non-smoking thin person. If you smoke and gain weight, you increase your death rate. Many thin smokers will say, "I can't quit smoking, I'll gain weight." Our data suggest that even if they gained a lot of weight (a 20 percent increase) but abstained from cigarette smoking, they would probably cut their death rate in half in the next 30 years!

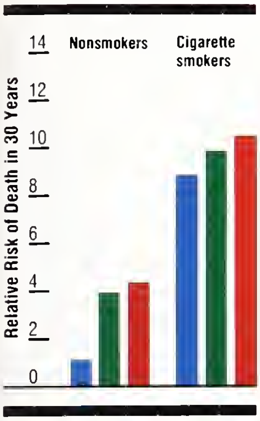


Figure 8.

Thirty-year relative risk of death for men aged 30 to 39 years at the first biennial examination. The blue bar represents men of desirable weight (Metropolitan Relative Weight, 100% to 109%); the green bar represents overweight men (relative weight, 110% or more) and the red bar represents obese men (relative weight, 120% or more).

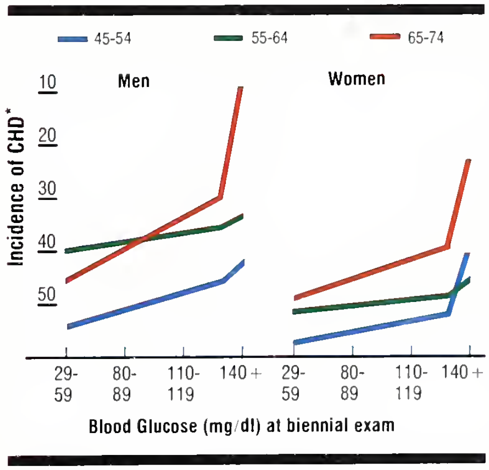


Figure 9.

Average annual incidence of coronary heart disease by blood glucose level.

*Data from Framingham study 16-year follow-up men and women 45-74 yrs. of age

Additional Risk Factors

Elevated blood sugar level increases the risk of all kinds of cardiovascular disease, including heart attack, stroke, congestive heart failure, and especially, peripheral vascular disease. This risk is especially apparent at the blood sugar levels seen in people with diabetes mellitus (Figure 9).

Exercise lowers the risk of coronary heart disease (Figure 10—next page); the more physically active you are, the lower the rate of heart attack. There are other CHD risk factors as well. Most major changes on an electrocardiogram, such as nonspecific ST- and T-wave changes, bundle-branch blocks, atrial fibrillation, and left ventricular hypertrophy, all greatly increase the rate of heart attack, stroke, and heart failure. High uric acid level, high hematocrit value, poor vital capacity (a measure of lung function), and high heart rate all increase the rate of cardiovascular disease. Some preliminary findings indicate that Type A personality or stress may increase risk. One of the newer risk factors found in Framingham is blood fibrinogen. The higher its level, the higher the cardiovascular disease risk.

Not everything proved to be a risk factor in the Framingham Heart Study. Coffee was not a risk, nor was sleep—other than at great extremes. Marital status was not a risk and, although it looked as if divorce or separation might lower risk, these data were not statistically significant. The precinct of town and educational status also proved not to be risks.

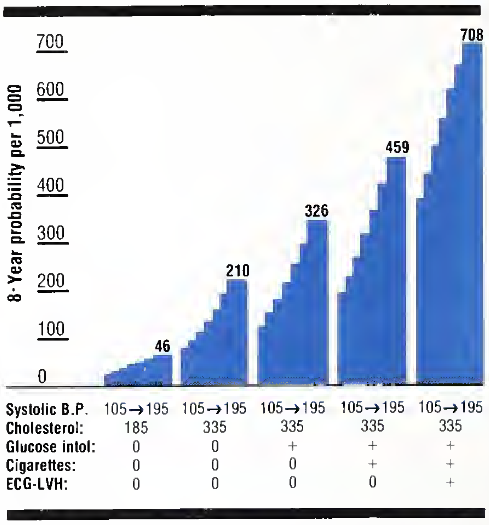


Figure 11.

Risk of cardiovascular disease and systolic blood pressure at specified levels of other risk factors.

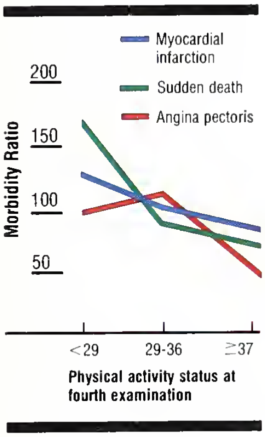


Figure 10.

Ten-year risk of developing coronary heart disease in men aged 30 to 62, and physical activity.

As Figure 11 shows, with few exceptions, the *combination* of risk factors for one person predicts risk better than does any *single* risk factor. In some respects, it is rather easy to identify most of the people in a town who are headed for a heart attack or stroke; they often display a constellation of risk factors.

During these 40 years of the Framingham Heart Study, the rest of the world was not idle. Many studies like Framingham were conducted in Albany, Chicago, Minneapolis, Tecumseh, San Francisco, and Evans County, Georgia. We began to see intervention studies done to lower blood cholesterol level, to reduce blood pressure, to halt cigarette smoking, and to increase physical activity. What did these studies show? Although some of the cholesterol-lowering studies produced findings discrepant with those from Framingham concerning cholesterol's effect on myocardial infarction, recent results from the Lipid Research Clinics Coronary Primary Prevention Trial are in agreement with Framingham's. Intervention trials in blood pressure showed that many of the effects of high blood pressure on cardiovascular disease can be reversed by lowering blood pressure. Stopping cigarette smoking can obliterate smoking's effect of increasing CHD risk. Exercise can even be shown to be effective in some settings.

During the past 22 years, Americans have seen the death rates for heart attack and heart disease fall. Better health habits, such as lowering animal fat and cholesterol intake, not smoking, and eating more fish and chicken have undoubtedly contributed to this. The Institute's High Blood Pressure Education Program, started in 1972, helped produce a dramatic fall in the stroke rate which was evident by 1975.

The Offspring Study

In 1970, the National Heart, Lung, and Blood Institute decided that it was time to study a second generation in Framingham for the effects of genes and heredity as they interact with society's environmental forces. A second generation also would reflect changes in our society as they might influence vascular disease and health. And so, incorporated into our study were the children, with their spouses, of the original members of the Framingham Heart Study. This study, known as the Offspring Study, involves about 5135 participants, who are just completing their 12th year of follow-up. Some good and some bad things appear to be happening in this second generation who, of course, are no longer children but are now aged 30 to 70. Blood cholesterol levels are slightly lower, blood pressures are lower, and men weigh more and women less than their fathers and mothers. Men smoke half as much as their fathers and women smoke more than their mothers and, for the first time in the history of the Framingham Study, more women are smoking than men. Children of parents with hypertension have higher blood pressures and male children of fathers with early heart attack have lower

HDL cholesterols. We have introduced many new technologies in the Offspring Study ranging from treadmills to Holter monitors which measure blood pressure. Echocardiographs were used to measure heart characteristics as well as to look inside the carotid vessels at the lesions themselves. It is hoped that the Framingham Heart Study can continue to monitor, in a free-living population, what is happening to the rates and factors associated with coronary heart disease. Recently, we have begun a small pilot study in the fourth generation to see how diet and exercise affect risk factors in very young children aged 4 to 8.

The Framingham Contest winners were honored at the May 1987 meeting of the National Heart, Lung, and Blood Advisory Council. From left to right: Carol Morse, Middle School Poster Winner; Dr. Castelli; Dr. Lenfant; Sarah Joiner, Elementary School Poster Winner; and Suresh Mulukutla, High School Essay Winner.

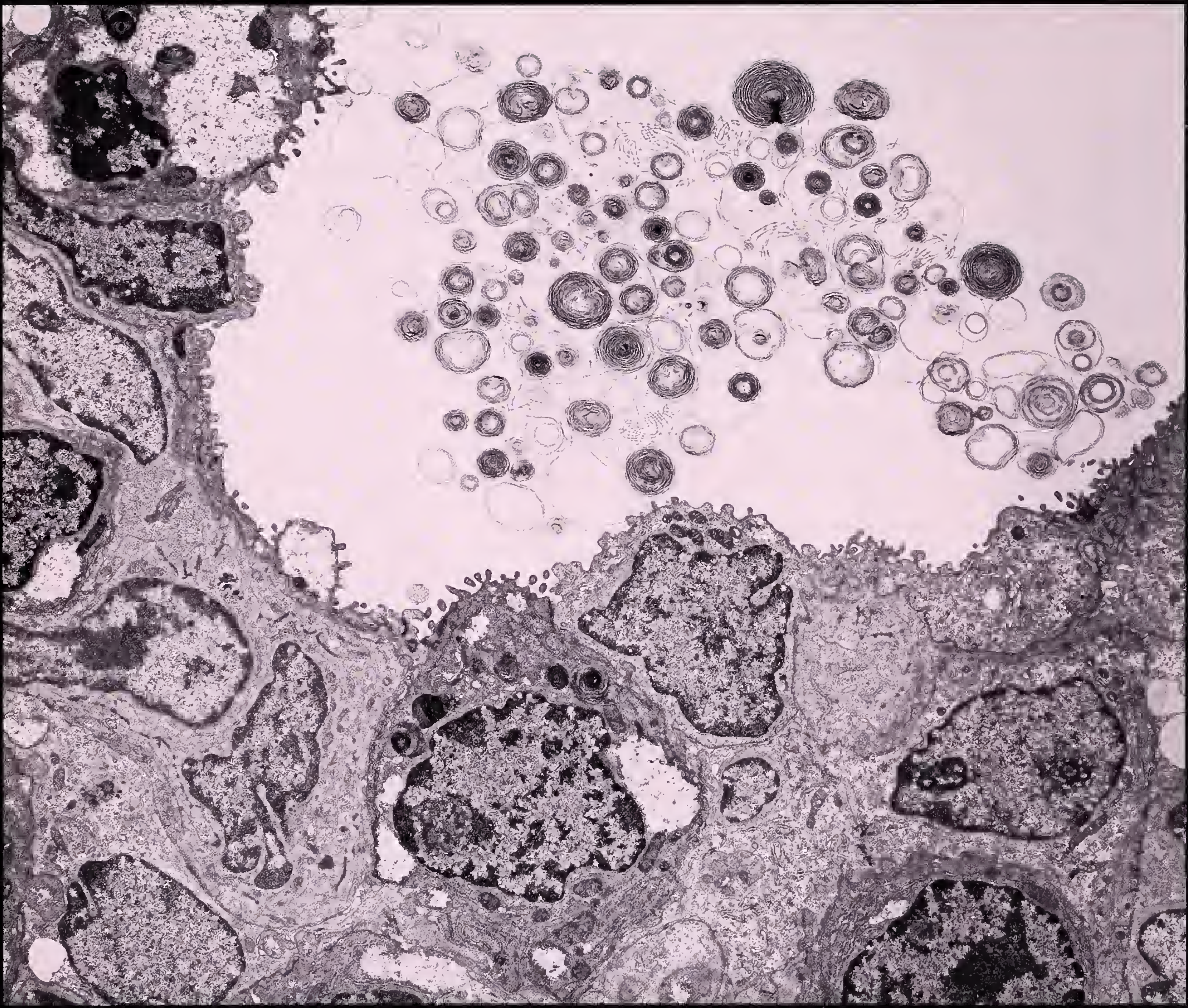
A Tribute to Framingham

But what of the people themselves in Framingham? They have faithfully endured all the examinations and intrusions into their lives that have been asked of them in the last 40 years. It was particularly gratifying in that context for those of us who have worked in Framingham that the NHLBI through the personal effort of its Director, Dr. Claude Lenfant, decided, as part of the centennial celebration of the National Institutes of Health and the 40th anniversary celebration of the NHLBI, to pay tribute to the people of Framingham for their efforts to teach the world about heart attack risk. In December 1986, leading cardiologists and cardiac surgeons, members of Congress and leaders of the Department of Health and Human Services, Public Health Service, NIH, and NHLBI came to the town of Framingham to tell the people how much their effort had helped them in their quest to conquer cardiovascular disease. Dr. Windom presented to Mr. King, Chief Selectman of the town, a tribute inscribed on parchment that thanked the citizens of Framingham for their dedication. An essay and a poster contest were held in all the schools and the winners were invited to the National Institutes of Health for a tour. They were also introduced by Dr. Lenfant to the members of the National Heart, Lung, and Blood Advisory Council at its May 1987 meeting and were presented awards for a job well done.

Most of us feel that great progress toward the prevention of cardiovascular disease has been made, particularly in the past 20 years. Unfortunately, there is a long way to go. One-half of the men and women who die in this country die from heart attack or stroke. The foundations for preventing these diseases have been laid. We need to intensify our campaigns to lower blood cholesterol levels, to lower blood pressure, to eradicate cigarette smoking, to lose weight, exercise more, and perhaps even to relax more. Much more will undoubtedly be learned to enhance our knowledge of how to do better, and even newer risk factors will appear; but, we have a start, and the 40 years of the Framingham Heart Study have contributed to it. The story of Framingham can help many people if the lessons are taken to heart.

William Castelli, M.D.
William B. Kannell, M.D., M.P.H.

Dr. Lenfant, Mr. John F. King, Chief Selectman of the town of Framingham, and Dr. Robert E. Windom, Assistant Secretary for Health, Department of Health and Human Services, at presentation of the tribute to Framingham, December 17, 1986.



Respiratory Distress Syndrome: A Mystery Unraveled

An electron micrograph shows the whorls of surfactant secreted into the airspaces of fetal lung. The surfactant is deficient in newborns with respiratory distress syndrome.

> **"How does it happen that the foetus continues in its mother's womb after the seventh month?, seeing that when expelled after this epoch, not only does it breathe, but without respiration cannot survive one little hour; whilst . . . if it remains in utero it lives in health and vigour more than two months longer without the aid of respiration at all."**
>
> William Harvey, 1651
> *De Generatione Animalium*

People speak of the "miracle" of birth with considerable justification. The transformation of a fetus, in a matter of seconds, into a living, breathing human being seems no less than miraculous when one considers the events that must take place. Respiration is the *sine qua non* of a successful entry into the world. Indeed, an infant is considered to be live-born at the moment when it draws its first breath.

The passage from fetal life to independent life by breathing air has fascinated scientists for centuries. One long-apparent fact is that timeliness is everything. In Harvey's day, an infant delivered before its time was doomed. Nothing was known about fetal lung development. During the ensuing 300 years, little was learned; the outlook for premature babies born in the 1950s was still dismal. Three decades later, however, premature newborns are surviving in record numbers. Respiratory distress syndrome (RDS), the bane of their existence, has relinquished its mystery and become one of the most completely characterized of all disorders.

Basic Concepts in Understanding Respiratory Distress Syndrome

RDS develops almost exclusively in premature infants usually within an hour or so after birth. The dominant feature of the disorder is atelectasis, collapse of the tiny air sacs (alveoli) in the lungs, which rapidly leads to respiratory failure. Typically, the infant shows many signs of a struggle for oxygen: breathing is rapid, the skin turns blue, the chest wall retracts noticeably with the effort of inspiration, and the infant grunts on expiration in an attempt to keep the alveoli from collapsing. Recovery, when it happens, usually occurs within a week. Until recent years, however, half of afflicted infants died.

Atelectasis is caused by the inability of the immature newborn to produce pulmonary surfactant, a unique substance that lines the alveoli and prevents lung collapse.

However, that knowledge did not come until 1959 and resulted from the confluence of seemingly unrelated discoveries from diverse scientific specialties. Meanwhile, physicians with a specific interest in the disorder pursued another, retrospectively fruitless, line of inquiry. As early as 1903, pathologists had taken an interest in examining the autopsied lungs of infants who had severe respiratory distress at birth and died shortly thereafter. They found the characteristic, translucent, homogeneous something dubbed hyaline (i.e., glassy) membranes, which gave the disorder its other commonly used name, hyaline membrane disease. For many years, researchers studied those hyaline membranes in the mistaken belief that they were the cause (rather than the result) of newborn atelectasis.

Our present understanding of the forces that conspire to produce atelectasis or its converse, alveolar stability, is grounded in the law that Laplace formulated in 1806 regarding surface tension. As applied to the lung, the law states that the pressure required to keep an alveolus expanded is directly proportional to the surface tension in the alveolar wall and inversely proportional to the diameter of the alveolus. In other words, it would take much more pressure to keep a small alveolus inflated than a large alveolus if both had the same surface tension. All of the alveoli in a given region of the lung are subject to about the same pressure. If they also have the same surface tension, the smaller ones will tend to collapse and push their air into the larger ones, which is exactly what happens in RDS. Infants who develop RDS lack surfactant, the alveolar lining fluid that reduces surface tension, and are thus at the mercy of Laplace's equation. On the other hand, infants with sufficient surfactant are not subject to this unstable condition. As an alveolus becomes smaller, the

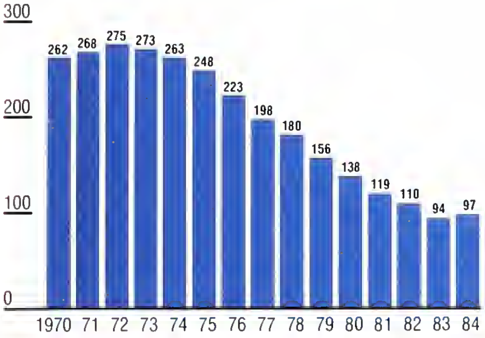
A nurse feeds breast milk through a tube to a baby girl born two months prematurely. About 40,000 infants per year in the United States are afflicted with neonatal respiratory distress syndrome.

Neonatal RDS: The Facts and Figures

Neonatal RDS strikes about 1 in a 100 babies, about 40,000 per year in the United States. It is responsible for approximately 20 percent of all newborn deaths and is the leading cause of death in the first 28 postnatal days. Despite major advances in detection and care, RDS is the third leading cause of infant death in the United States, according to the National Center for Health Statistics. About 10 percent of RDS victims will die, most within the first 4 days of life. The incidence varies inversely with gestational age: it increases with the extent of immaturity of the infant and is almost never present in infants born at 38 weeks of gestation. The encouraging news is that the mortality from RDS has decreased dramatically during the past 15 years, from approximately 10,000 infants in 1970 to 4,000 in 1984.

surfactant becomes more concentrated and surface tension is reduced. Conversely, as an alveolus becomes larger, the surfactant is spread more thinly on the fluid surface and surface tension increases. This special characteristic of surfactant prevents lung collapse by ensuring that all of the alveoli in a given region of the lung remain approximately the same size. It also helps keep alveoli open during exhalation by maintaining a low surface tension at small volumes.

Infant Mortality from Neonatal RDS (Hyaline Membrane Disease) per 100,000 live births in the United States, 1970-1984



The rate of death from neonatal RDS has declined by almost two thirds between 1970 and 1984, the most recent year for which data are available. The most dramatic decline occurred in the mid-1970s, a time when continuous positive airway pressure ventilation and improved treatment strategies for premature infants came into widespread use.

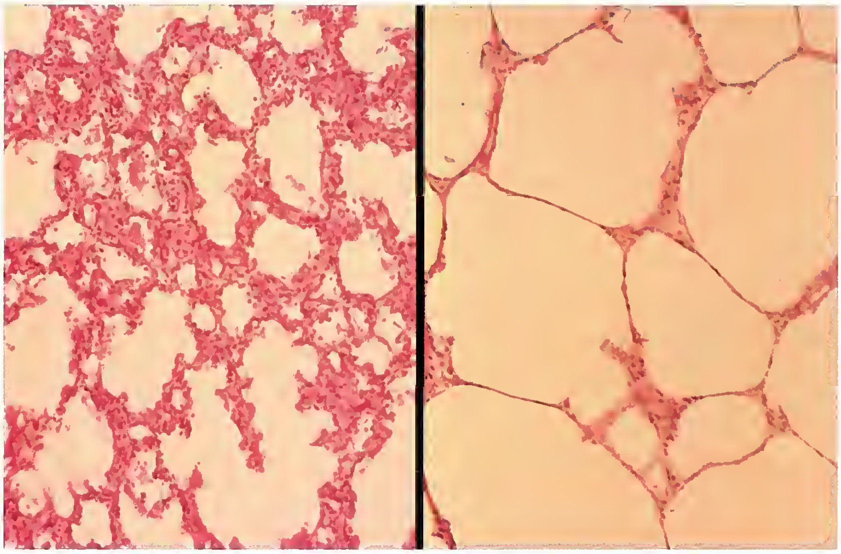
Until this century, the lung's remarkable ability to expand and retract had been attributed entirely to the elasticity of the tissue. Lung retraction was associated with surface tension in 1927, when it was demonstrated that surface tension accounted for the major portion of lung retraction. Unfortunately, the significance of surface tension as a factor to be counteracted by a newborn's first breath was not appreciated at that time.

Twenty years later, measurements of the minimum pressure needed to inflate the autopsied lungs of stillborn infants revealed that it took twice as much pressure to expand the lungs with air as it did with saline because of the presence of surface tension at the tissue-air interface. When amyl acetate (which reduces surface tension) was introduced into the lung, the pressure needed to inflate the lung with air was reduced. It was suggested that adding surface tension-reducing agents to the inspired air of newborns suffering from atelectasis might relieve their respiratory distress; this, too, went unheeded. Most students of RDS were still chasing hyaline membranes.

Expansion of Knowledge

Not until the 1950s was this important work on surface tension and the lung applied to the problems of RDS for, until then, there was no knowledge of how appropriate surface tension is maintained and even less understanding of what immature infants had or lacked that made their lungs collapse. In 1954, the observation was made that certain lung cells (granular pneumonocytes) appeared to secrete a watery, mucoid film that lined the alveolar layer and possibly functioned to maintain favorable surface tension. That same year, estimates of the total surface area of alveoli produced results that would make sense only if the lung lining consisted of a highly surface-active material.

More evidence accumulated the following year, when foam extracted from the lung was shown to be long lasting and stable. It was concluded that this unusual behavior resulted from low surface tension and was derived from some insoluble layer lining the alveoli. It subsequently was discovered that this lining layer appears only late in the fetal life of the guinea pig—the first hint of this work's relevance to the problems of premature infants. In 1956, it was discovered that when lung extracts were compressed, their surface tension fell, whereas when expanded, surface tension rose. Here was evidence for the unique property of alveolar tissue, the antiatelectasis factor described above that keeps alveoli open during expiration. Building on this work in 1959, a team of researchers published a paper that established the case for surfactant. They compared the surface tension of the lungs of babies who died of RDS with that of the lungs of newborns and older persons who died of other causes. When the lung tissue was compressed, surface tension was low in the comparison groups but remained high in the RDS babies. The problem became clear; newborns with RDS suffered alveolar collapse because they lacked the surfactant necessary to keep their lungs open.



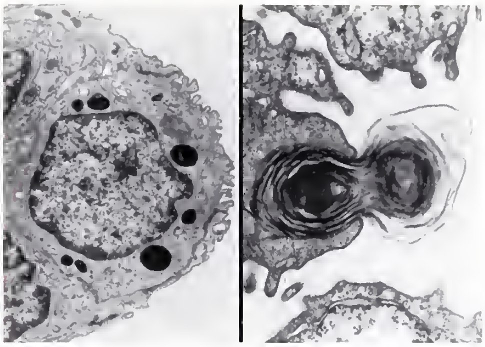
Normal lungs of full-term and premature infants who did not have respiratory distress syndrome (RDS) are characterized by open alveolar spaces and thin alveolar walls (right). In contrast, lungs of infants with neonatal RDS show greatly thickened alveolar walls and collapse of some alveoli (left).

Following that key discovery, new knowledge accumulated rapidly as the field attracted researchers from a variety of disciplines. Many exciting challenges became apparent: to characterize surfactant, identify its source, find a method to hasten its arrival or replace it in immature infants, and discover ways to keep babies alive until they developed their own surfactant. Fortunately, these questions came when new disciplines and techniques were becoming available to answer them, including modern lipid and protein chemistry, high-resolution electron microscopy, cell biology, amniocentesis, and sophisticated bioengineering, which made neonatal intensive care a reality.

Surfactant Biology

In 1954, a particular cell type found in the lining of the lung alveoli, the type II alveolar epithelial cell, was first recognized to be the possible source of surfactant. Researchers used the electron microscope to examine this cell, which we now know is unique to the lung. They found unusual structures within the cells that had a lamellar form (i.e., they appeared to be composed of many concentric layers, much like an onion). Other investigators observed that these lamellar bodies were absent from the lungs of very premature animals and did not appear until the time in gestation when the surfactant was present.

The NHLBI took an active role in supporting the anatomical, physiological, and biochemical studies conducted in the late 1960s and throughout the 1970s that



Type II cell of rat lung (left). The nucleus, mitochondria, and the lamellar bodies of the cell are shown. As the cell responds to signals that more surfactant is required on the alveolar surface, the contents of the lamellar bodies are released into the airspace (right).

sought to discover the chemical nature and regulation of surfactant. By 1972, pulmonary surfactant had been isolated, and the lipid portion of surfactant had been described almost completely. It was clear that a substance called dipalmitoyl lecithin was the active ingredient responsible for its highly surface-active properties. In the following year, investigators identified a surfactant-associated protein that was unique to the lung.

It became clear that, because the lung contains so many different types of cells, efforts to determine the exact nature of the cellular control of surfactant were being impeded. The Institute launched a new program in 1973 to encourage the isolation and characterization of lung cells. By then, there was much evidence from sophisticated biochemical and anatomical experiments that the type II cell manufactures and stores surfactant. Studies were undertaken to isolate type II cells and grow them in the laboratory. For the first time, direct evidence that these cells were the source of the surfactant could be obtained. The realization that surfactant is made by cells only found in the lung sparked a virtual explosion of research activity and marked the beginning of an exciting new field of lung research. The lung was viewed not only as a respiratory organ but also as an organ that had active metabolic functions vital for life. A major new effort to elucidate the metabolic, or nonrespiratory, lung functions had begun.

Many scientists provided important new insights and clues to the metabolic pathways of surfactant synthesis and regulation of its secretion. The studies with isolated cells, in conjunction with biochemical analyses of surfactant, and investigations into the physiologic role of surfactant in the living organism laid the groundwork for the dramatic improvements that have occurred in the detection, prevention, and treatment of infants with RDS.

The excitement and sense of accomplishment resulting from the work of the last twenty years continue to this day. New opportunities made possible by the recent advances in molecular biology and genetics have resulted in major contributions to our understanding of surfactant biology. This work has great promise for new advances in the care of infants with RDS. Findings from the basic sciences have contributed in a number of important ways to the favorable outlook for infants at risk of RDS.

Prenatal Testing

The first important step in the development of a prenatal test to detect infants at risk for RDS was the ability to identify infants before birth who lacked sufficient surfactant to breathe on their own. Amniocentesis was developed in the 1930s. However, at that time and for many years after, it was thought that amniotic fluid flowed into the lungs, rather than out of the lungs; therefore, the procedure was not considered useful in assessing fetal lung development. The first separation of lung fluids from amniotic fluid was achieved in 1963. In doing so, the researchers provided proof that lung fluids flow outward and contain pulmonary surfactant.

The first clinical prenatal test to diagnose immature lungs was devised in 1971 and involved measuring the ratio of lecithin (a major component of surfactant) to sphingomyelin (a nonsurfactant lipid found in the lung) in the amniotic fluid. A high lecithin/sphingomyelin (L/S) ratio indicated sufficient surfactant, whereas a low ratio meant the baby would have RDS. Knowing this before delivery permitted physicians to postpone the delivery, if possible, or to transfer the mother to a hospital with intensive care capabilities before the baby was born.

The shortcoming of the L/S ratio test was that it required a laboratory, sophisticated analytic procedures, and about 2 hours to perform. The following year, a faster, simpler, and cheaper test was devised. This new test was based on the observation, made two decades earlier, that surfactant produces a very stable foam. The "shake test" that resulted could be done at the bedside and involved mixing equal parts of amniotic fluid and ethyl alcohol, shaking the mixture, and checking for long-lasting bubbles.

Prevention of Respiratory Distress Syndrome

Now that it was possible to predict immature lungs prenatally, the question arose: Is there any way to hasten the arrival of surfactant before the baby is delivered? It was discovered in 1969 that lambs delivered prematurely after infusions of corticosteroids had mature lungs. The suggestion that corticosteroids accelerate the appearance of surfactant was soon confirmed and extended by morphologists and biochemists studying surfactant production, and the efficacy of the treatment was demonstrated in lambs and rabbits.

Results of a clinical trial conducted in New Zealand in 1972 provided evidence that corticosteroids administered before premature delivery could reduce the incidence of RDS in human newborns. A multicenter, randomized, double-blind, collaborative clinical trial was initiated by the NHLBI in 1976 to determine the efficacy of treatment and to identify any short- or long-term adverse effects on the mother and infant. The study population comprised expectant mothers between 26 and 37 weeks of gestation (40 weeks is full-term) who entered the hospital at high risk for premature delivery and whose L/S ratio indicated fetal lung immaturity. Patients were assigned randomly to receive either dexamethasone phosphate (a corticosteroid) or a placebo over the course of 48 hours before delivery. The results of the trial confirmed earlier work and demonstrated that the prenatal administration of corticosteroids is effective in reducing the overall incidence of RDS. However, they indicated that the magnitude of the treatment benefit might depend upon the sex, race, and other characteristics of the infant and mother. Follow-up studies revealed that no detectable adverse effects of the steroid treatment were apparent for the first 3 years of life.

Research on premature sheep was the key to the discovery that corticosteroids can accelerate lung maturation before birth.

These findings gave physicians an important tool to use in the prevention of RDS. They also stimulated further work on the role that hormones play in the production of surfactant. Studies in a number of mammalian species, including humans, rats, mice, and rabbits, have shown the delayed appearance of surfactant in male fetuses. The lack of effect of dexamethasone on male fetuses observed in the clinical trial reinforced the point that sex differences exist in surfactant production. Currently, the lag in male fetal surfactant production is believed to be caused by the presence of male hormones (androgens) that inhibit surfactant synthesis. This knowledge has provided some clues that may help another group of babies at high risk of developing RDS: the offspring of diabetic mothers. Recent work has shown significant elevations in the levels of two male hormones in the amniotic fluid of diabetic women. There is speculation that in diabetic pregnancies, exposure of the fetal lung to elevated levels of androgens may inhibit surfactant production. Consistent with this was the observation that the amniotic fluid of male fetuses of diabetic mothers had the highest concentration of testosterone and the lowest L/S ratio. If androgens prove to be important inhibitors of fetal lung maturation, hormonal therapies may be effective in preventing RDS.

Investigators also are looking at the role of thyroid hormone in the stimulation of surfactant production. There is evidence that thyroid hormones and corticosteroids may act synergistically to stimulate the synthesis of surfactant lipid. A recent study showed that when thyroid hormone was administered in conjunction with dexamethasone to fetal rats, it produced an effect in both males and females that exceeded the effect of dexamethasone alone. If studies in humans bear this out, it is possible that a thyroid hormone-dexamethasone combination may prevent RDS in males.

Progress in Treatment: Intensive Care
Although advances in prevention have spared many newborns the problems of RDS, the syndrome still develops in thousands of babies; about 40,000 infants per year in the United States are affected by RDS. The great improvements in survival that have occurred during the past 25 years are attributable to the backbone of RDS treatment: neonatal intensive care. Before 1965, there were no intensive care facilities for newborns, but technological innovations occurring about that time spurred the development of many at major medical centers. Since the mid-1970s, the regionalization of perinatal health care has become standard. Mothers in premature labor and tiny newborns are transported routinely to tertiary care facilities equipped to handle their special problems.

Advances in neonatal intensive care have been dramatic. Transporter incubators are almost a nursery on wheels, complete with oxygen, heat, and devices for monitoring heart rate.

The objective of RDS treatment is to support the newborn in a manner that facilitates the production of pulmonary surfactant and consequent recovery from RDS. Before 1969, many types of assisted or mechanical ventilation used in adults were tried in newborns with RDS, but mortality remained high. Most babies were treated with some form of intermittent positive pressure (i.e., pressure only during inspiration), and their lungs almost invariably collapsed on expiration. This alveolar collapse placed a severe strain on the newborn's respiratory muscles. Further, it was shown that after collapse, the alveolar surface film ruptured on reexpansion and did not reform, thus straining the infant's already-limited capacity to produce adequate amounts of surfactant.

In 1969, investigators supported by the NHLBI developed continuous positive airway pressure (CPAP), which involved maintaining a steady pressure throughout the entire respiratory cycle. The results were dramatic: the alveoli remained open on expiration, surfactant production caught up with demand, and many babies survived. An added benefit was that CPAP also was found to be effective using lower concentrations of oxygen than were other methods, thereby reducing the toxic effect of oxygen on the lungs.

Concurrently, great advances were made in the technology used to monitor blood gases in newborns. The "miniaturization" of equipment and techniques used for adults permitted frequent, safe, and accurate monitoring of the oxygen level in arterial blood. The ability to maintain blood

At six weeks of age, Adrienne weighs only 800 grams (1 pound, 12 ounces). Knowledge of the nutritional requirements of babies as small as Adrienne is still incomplete, and careful monitoring is essential.

oxygen at an appropriate level greatly reduced the occurrence of blindness from retrolental fibroplasia, which had been a common complication of oxygen therapy.

A third area of advance in recent years is nutritional support for premature infants. The typical baby with RDS is born without adequate energy stores and is usually too ill to nurse. Moreover, a tremendous amount of energy must be expended just to breathe and to maintain body temperature. Recent nutritional studies have identified key nutrients and improved methods of providing them to prevent loss of protein and to meet the caloric requirements of the infant with RDS.

Modern intensive therapy has ensured that most babies will survive RDS. However, it has been estimated that more than half of neonates with RDS will manifest some complication of intensive therapy. A major respiratory complication is bronchopulmonary dysplasia, a chronic lung disorder that was first recognized in 1967. The condition involves abnormalities in the architecture of the lung that lead to poor gas exchange and, in many instances, progressive respiratory failure. Much research is being done on bronchopulmonary dysplasia, but many questions remain to be answered.

High frequency ventilation (HFV) is now a new form of therapy being evaluated to assess its usefulness in reducing the complications of treatment for RDS. HFV is a method of ventilation that uses several times the normal ventilation rate. (For a newborn, whose spontaneous respiratory rate is 30 breaths per minute, a rate of 120 breaths per minute would be HFV.) Because HFV operates at low pressures, it may reduce the incidence of air leaks and other side effects of conventional treatment. A multicenter, randomized, collaborative clinical trial sponsored by the Institute is underway to determine if HFV is more effective than conventional ventilating machines in treating premature babies and reducing the incidence of chronic lung diseases.

Katie weighed only 530 grams (1 pound, 3 ounces) at birth and had neonatal RDS. She was treated with a surfactant preparation isolated from human amniotic fluid. She is shown here at fourteen months of age.

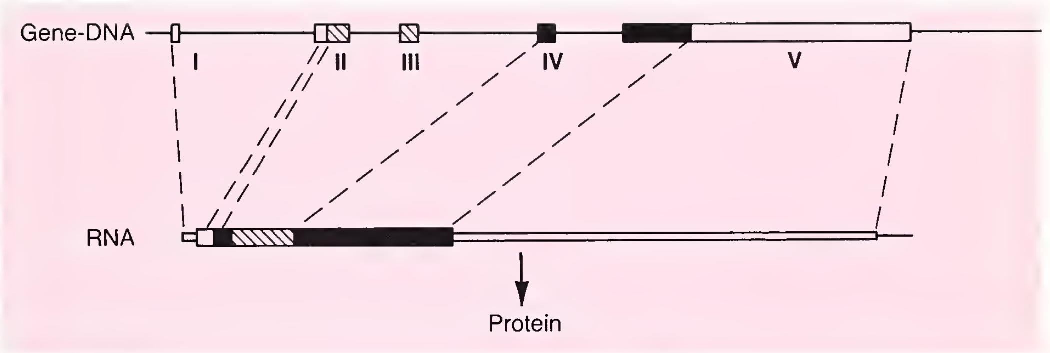
Progress in Treatment: Surfactant Replacement

Ever since it was discovered that RDS results from a deficiency of surfactant, the development of an effective, safe replacement therapy has been a dream of clinicians and basic researchers. Attempts were made in the 1960s to introduce surfactant components into the lungs of animals and infants with respiratory distress, but the results were inconsistent. Real progress awaited the elucidation of the physical and chemical characteristics of surfactant and the discovery of an effective means of delivering surfactant to the lungs. Natural surfactant was isolated from animal lungs and had beneficial effects when instilled into the tracheas of premature animals with respiratory distress. The first successful report of replacement therapy in human infants came from Japan in 1980. Ten infants with severe RDS were given a surfactant preparation isolated from cow lung, and the results were very encouraging.

Since that time, several different preparations have been developed. Surfactant from natural or animal sources is available from cows and pigs, and a human surfactant can now be obtained from amniotic fluid. Other preparations are natural surfactants supplemented with additional chemical compounds and synthetic surfactants that are made in the laboratory. These various surfactants are being tested in clinical trials throughout the United States, Canada, and Europe. The NHLBI supports many of these investigations. It is encouraging that several studies published to date suggest that treatment of RDS with surfactant replacement improves survival and reduces the incidence of bronchopulmonary dysplasia. The results also suggest that the therapy is safe and without significant toxicity. Further careful study is needed before the effectiveness and long-term safety of surfactant therapy can be determined.

Back to Basics and On to the Future

We already have alluded to the new opportunities afforded by the application of molecular biology and genetics to the surfactant field. In 1985, NHLBI-supported



The gene for the major human pulmonary surfactant-associated protein has five coding regions called exons that are shown as boxes numbered I through V. The structure of the messenger RNA for the surfactant protein is also shown. The dotted lines connect the exons with corresponding regions on the messenger RNA. The genetic messages are then translated into the protein. Molecular biological methods are enabling scientists to probe the genetic regulation of surfactant.

investigators announced the isolation and characterization of the gene that encodes the major surfactant-associated protein. Several surfactant proteins have been isolated and characterized, and their role in facilitating the functions of surfactant are being elucidated. Molecular biology methodology enables scientists to probe the genetic regulation of surfactant. Recent work has localized the gene for the major surfactant protein to a particular human chromosome.

Researchers have introduced the human surfactant gene into cells in culture that can now manufacture the human surfactant proteins. A new avenue of research is the development of a bioengineered surfactant replacement therapy that would eliminate the concern of possible allergic reactions resulting from introducing a foreign surfactant protein into sick, immature infants. Whether such a preparation can be devised that will be as effective as the natural surfactants remains to be seen. Other questions for future study include determination of whether abnormalities of the surfactant gene are important in neonatal RDS. If so, a more accurate and specific prenatal screening test could be devised to detect and better treat infants at risk for RDS. Scientists also speculate whether surfactant therapy might prove beneficial in certain lung diseases of adults that are characterized by surfactant deficiency.

The remarkable advances in surfactant research that have occurred in the past few decades have saved the lives of many babies and resulted in a new understanding of a complex and unique biological system. The unfolding of basic and clinical aspects of the surfactant story has truly captured the imagination of pulmonary investigators. Their discoveries continue to challenge researchers to learn more, not only about surfactant, but also about the lung as a whole, both during normal development and prematurity.

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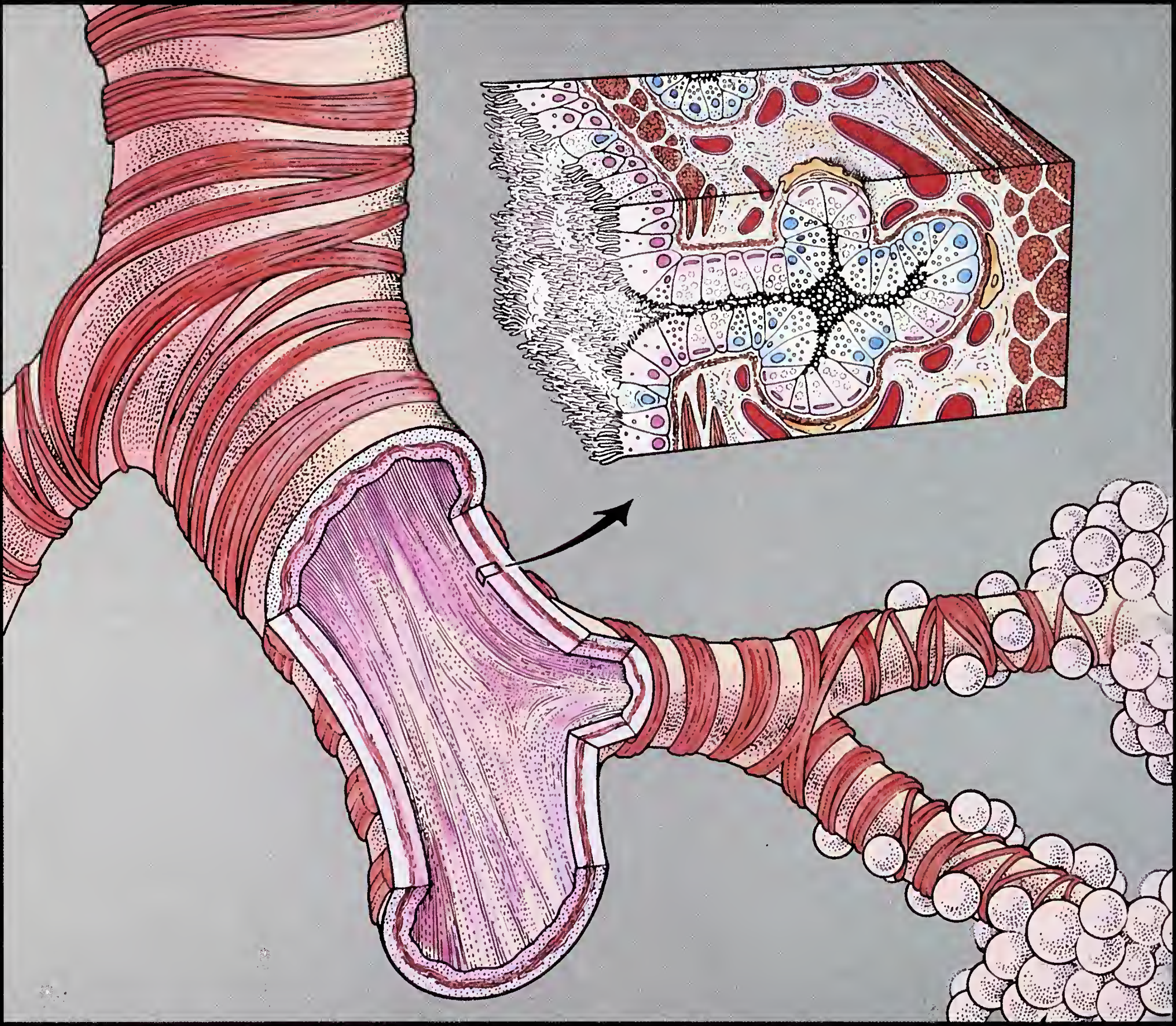


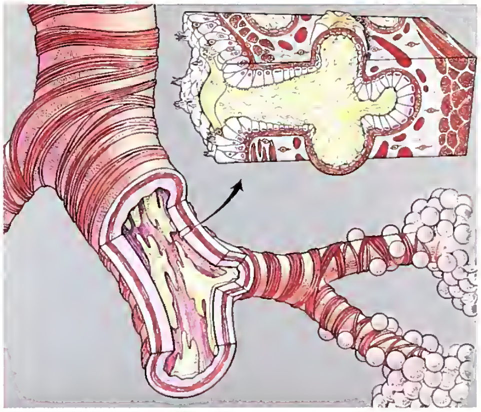
Chronic Obstructive Pulmonary Disease

Twelve to 15 times every minute, a typical adult inhales 1 to 2 pints of air. This life-sustaining process occurs involuntarily, as well it must. If respiration is prevented, death is inevitable within minutes. Because it is involuntary, we are generally not conscious of the process of respiration. This is not the case with individuals who have chronic obstructive pulmonary disease, a disorder that affects more than 10 million individuals in the United States. People who have chronic obstructive pulmonary disease (COPD) are very aware of the process of breathing because they must work harder to breathe.

The function of the lung is to transfer oxygen, a critical fuel for all life-sustaining processes, from air to blood and, in exchange, to transfer carbon dioxide, the waste gas of cellular metabolism, from blood to air. The anatomy of the lung is that of an ideal gas exchanger. The airways (bronchial tree) provide the branched conducting tubes that direct air to and from the air sacs (alveoli), the actual site where air and blood are brought into close apposition to provide efficient gas exchange. In COPD, the flow of air in the airways is impaired. Consequently the affected individual has to expend more effort to exchange gases.

Impact of COPD

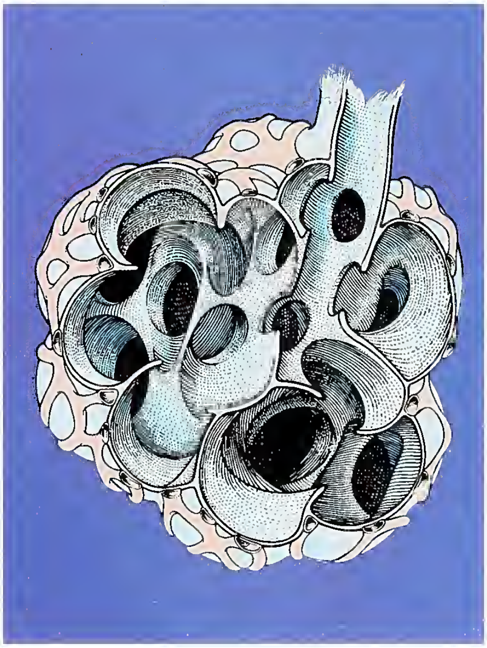
A slowly developing, irreversible, and generally progressive disorder, COPD actually includes several different, but often coexisting, processes, including chronic bronchitis, asthmatic bronchitis, and emphysema. Chronic bronchitis is a disease of the airways, the branching tubes that bring air from the pharynx to the air sacs, or alveoli. In chronic bronchitis, there is irreversible



(Left) Normal bronchial tree. The inside lining (insert) is composed of cells that secrete mucus and cells with hair-like projections (cilia) that sweep mucus out of the lung, thus providing a continual cleansing action that clears inhaled particulates and bacteria from the lung.

(Right) The bronchial tree in chronic bronchitis. The airway wall is thickened. A close-up view (insert) shows the mucus gland filled with thickened mucus which is being secreted into the airway lumen. There is a loss of the normal ciliated airway cells and an increase in the number of mucus-producing cells. Such a deranged airway cannot provide the normal path for airflow in and out of the lung and has difficulty defending the lung from inhaled infections.

narrowing of the airways, accompanied by excess production of mucus, causing the affected individual to cough up sputum on a chronic basis. Asthmatic bronchitis is similar to chronic bronchitis but, intermittently, there is a superimposed reversible worsening of the airway obstruction. In contrast to both chronic bronchitis and asthmatic bronchitis, emphysema affects the airways only indirectly. The major problem in emphysema is irreversible destruction of the walls of the alveoli. Consequently, there is less mechanical force available to move air in and out of the lungs and to keep the airways open—the result is airway collapse and obstruction to airflow. When there is a mixture of chronic bronchitis, asthmatic bronchitis, and emphysema—as occurs in many affected individuals—the disease process is called COPD. If the different processes occur separately, they generally are referred to by the specific entity.

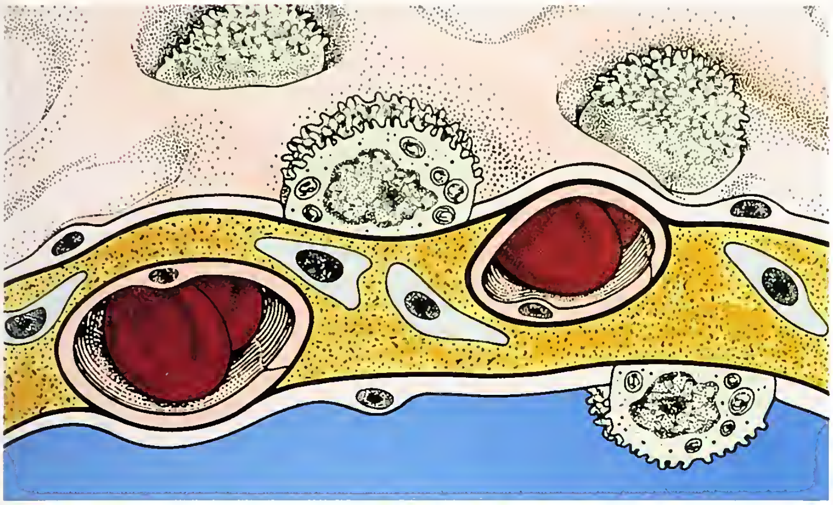


The normal lower respiratory tract. The small airways branch into the alveoli, beehive-like structures that are the site of gas exchange.

Morbidity from COPD results from the progressive loss of lung function. Affected individuals are short of breath, at first with exercise, and later at rest as well. There is an increased incidence of lung infections, including pneumonia, particularly for those in whom bronchitis and asthmatic bronchitis dominate the illness. Consequent to these problems, individuals with COPD have limitations of their activity levels. The illness causes more days away from work, more days at home in bed, increased visits to physicians, and increased numbers of hospitalizations. Death from COPD usually occurs following long periods of disability.

The impact of COPD on society is enormous. It is the fifth leading cause of death in the United States. In 1985, approximately 70,000 Americans died from COPD, and it was a contributing cause of death in another 80,000. In the United States alone, the economic costs are staggering. In 1982, $3.2 billion were spent directly on health expenditures for COPD, and the illness indirectly cost society $2.4 billion because of limitations placed on affected individuals by the disease.

Despite these statistics, discoveries over the past 40 years have markedly changed the manner in which COPD is conceptualized, evaluated, and treated. Most strikingly, epidemiologic studies have



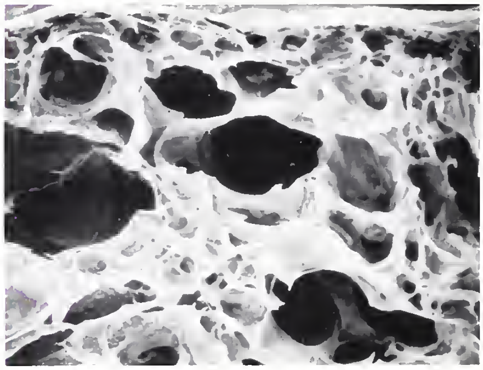
The normal alveolar wall. The walls of the air sacs are thin, fragile structures that bring air and blood into close approximation, thus providing efficient gas exchange. Shown are capillaries with red blood cells passing through the alveolar wall.

defined the major cause of COPD; new physiologic tests now permit early detection of the disease process; the development of bronchodilators, drugs that help keep airways open, and antibiotics, drugs that suppress infection, have improved the quality of life for affected individuals; chronic oxygen therapy has extended the life span of patients with advanced COPD; and the discovery of alpha-1-antitrypsin deficiency, an hereditary disorder, has led to an understanding of the biologic processes that lead to emphysema and how these may be reversed by therapy.

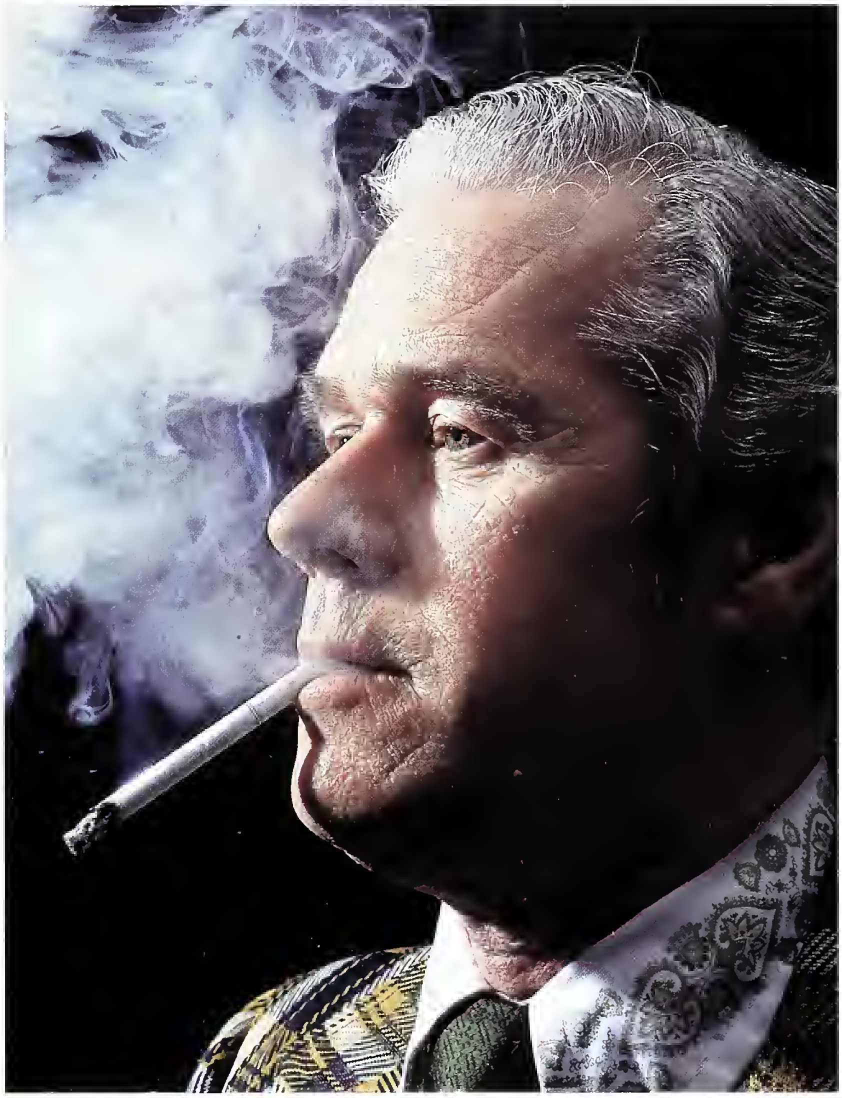
The Epidemiology of COPD—The Cigarette as the Causative Agent

Over the past 100 years, a variety of factors have been implicated as increasing risk for the development of COPD. Among these, the most tenable hypotheses included air pollution, susceptibility to reactive airway narrowing (i.e., asthma), the consumption of alcohol, an increased susceptibility to allergies, a history of childhood respiratory problems, chest infections, occupation, socioeconomic status, hereditary factors, and cigarette smoking. In the past 40 years, all of these have been subjected to rigorous epidemiologic study, a scientific discipline that evaluates the relationships of various factors determining the frequency and distribution of disease. Over and over, these studies have led to the same conclusion—that cigarette smoking is the major cause of COPD.

The concept of an association between cigarette smoking and COPD is not new. Studies in the late nineteenth century reported increased respiratory problems in smokers compared with nonsmokers, but it took the tools of modern epidemiology to prove the association. The results of these studies are overwhelming and have convinced the entire scientific community of the association of cigarette smoking and COPD. Evidence leading to such strong conclusions comes from studies on the association of various risk factors with respiratory symptoms and impairment of lung function, deaths attributable to COPD, and evaluation of samples of lung tissue.



Scanning electronmicroscope picture of the lower respiratory tract in emphysema. Normal beehive-like structures at the alveoli are lost secondary to destruction of the alveolar walls. The results are large spaces that do not function in normal gas exchange.



Cigarette smoking is the major cause of chronic obstructive pulmonary disease in the United States.

Based on studies of populations, it has been demonstrated that cigarette smoking is the major cause of respiratory symptoms and impairment of lung function in the United States. Of all factors evaluated, cigarette smoking is, by far, the major predictor for the development of COPD. Within a few years after beginning to smoke, cigarette smokers experience a higher prevalence of respiratory symptoms and abnormal lung function. These symptoms and lung function abnormalities increase with the duration of smoking and are much greater in heavy smokers than in light smokers.

Although historically males have had a higher incidence of COPD than have females, this is probably because, in the past, males smoked more than females. Importantly, evidence from several studies has demonstrated that the ability to exhale air rapidly (the major physiologic abnormality in COPD) declines at a greater rate over time in smokers compared with nonsmokers.

Not only is there clearly an increased morbidity associated with cigarette smoking, but prospective and retrospective studies consistently have shown an increased mortality from COPD in cigarette smokers compared to nonsmokers. The risk of death from COPD is linked directly to the number of cigarettes smoked daily and the number of years the individual has maintained the smoking habit.

By analyzing pieces of lung tissue obtained at surgery or autopsy from cigarette smokers and comparing them with lung tissue of nonsmokers, it became clear that cigarette smoking caused abnormalities in the lung characteristic of COPD. These studies show that cigarette smoking causes abnormal changes in the large airways, small airways, and in alveoli. In the large airways, there are increases in the size and number of glands that produce mucus, consistent with the knowledge that cigarette smoking is associated with an increased incidence of phlegm production. In the small airways, smoking causes inflammation and narrowing and, in the air sacs, cigarette smokers have evidence of destruction, the hallmark of emphysema.

The conclusions of these studies are best stated in the summary of the 1984 Report of the Surgeon General on the Health Consequences of Smoking:

> *Cigarette smoking is the major cause of chronic obstructive lung disease in the United States for both men and women. The contribution of cigarette smoking to chronic obstructive lung disease morbidity and mortality far outweighs all other factors.*

The overwhelming conclusion is inescapable. Without cigarette smoking, COPD would have, at most, a minor impact on the health of society.

Adaptation of Physiologic Principles to Human Disease—Early Detection of COPD

It is a central tenet of clinical medicine that if disease can be detected early, there is a greater chance of reversing the disease process or at least slowing it. In the 1940s and 1950s, methods were developed that defined the physiologic abnormalities of COPD. Once these were established, the search began for lung function tests that were sensitive to the changes in the lung that characterized this disease.

As the term suggests, COPD is characterized physiologically by obstruction to airflow. Although there are some changes to airflow during inspiration, the hallmark of the disease is obstruction to expiratory flow. The problem for early detection is that the first changes that occur in COPD are in the small airways. Because of the anatomy of the lung, the major resistance to expiratory airflow is in the large airways. Since the small airways contribute less than 10 percent of the total resistance to airflow during expiration, even marked thickening of the small airways alone has little effect on overall resistance to airflow and hence on expiratory airflow as detected at the mouth. Consequently, disease confined to the small airways was difficult to detect with conventional lung function testing equipment.

The realization of this problem in the 1960s led to intensive study by NHLBI investigators to develop tests that would be sensitive to changes in peripheral airway obstruction. The problem was essentially one of engineering, in which the sophisticated knowledge of physiologic principles was adapted to clinical medicine.

Lung function testing. Sophisticated instrumentation is now available to detect loss in lung function.

The first method to detect so-called small airways disease required the subject to breathe radioactive xenon-133 gas. When there was increased resistance in the small airways, the radioactive gas remained in the lung for periods longer than normal. Other early approaches utilized comparisons of the amount of air exhaled with measurement of esophageal pressure (a measurement equivalent to the pressure between the chest wall and the lung) while the subject took small, rapid breaths. By stressing the lung in such a fashion, it was possible to demonstrate that abnormalities in small airways limited these dynamic volume-pressure relationships.

While sensitive, thus fulfilling the criteria for early detection of small airways disease, both methods were cumbersome—radioactive gas in one instance and the necessity of placing a small balloon in the esophagus in the other. What was needed was an easily performed and reliable test that could detect early COPD. One such approach was the adaptation of an older test, the so-called single breath nitrogen test. From physiologic principles, investigators argued that the airways in portions of the lung occupying the lower part of the chest closed down during expiration before their counterparts in the lung in the upper chest. This differential timing of airway closure causes a change in the percentage of nitrogen exhaled after taking one breath of oxygen. The point during exhalation at which this occurs—called the closing volume—occurs earlier in COPD compared to normal individuals. The increased closing volume in COPD was shown to correlate with the morphologic changes in the small airways characteristic of this disorder. Another approach based on physiologic principles was to record the rate of airflow while the subject breathed out as rapidly and as hard as possible. This was repeated while the individual then breathed a mixture of helium and oxygen—a mixture lighter than air. By comparing the results of the two tests, investigators concluded that the differences were related to airway narrowing.

One of the important sequelae of the development of these sensitive tests is their use in detecting early abnormalities in the airways of young individuals who smoke cigarettes. In this regard, studies have demonstrated that in young cigarette smokers who have abnormal tests of small airway function, but otherwise are normal, discontinuance of the smoking habit results, over time, in reversal of the functional airway abnormalities. Thus, methods are now available to detect early abnormalities in airway function, early enough to permit reversal before COPD becomes chronic and potentially disabling. Such tests are now available to pulmonary physicians throughout the country.

Bronchodilators — Opening the Closed Passage

The three components of COPD—chronic bronchitis, asthmatic bronchitis, and emphysema—each have a different pathogenesis and, consequently, there are different approaches to their therapy. Asthmatic bronchitis is unique among the three in that obstruction to airflow associated with it is, at least in part, reversible. For those individuals with COPD in which asthmatic bronchitis plays a dominant role, this reversible component is one aspect of the disease that is vulnerable to therapy with pharmacologic agents that act to dilate the narrowed airways.

Typically, an individual affected with COPD dominated by asthmatic bronchitis will have a baseline level of respiratory insufficiency that intermittently gets worse. This intermittent deterioration occurs over hours and sometimes days and frequently is accompanied by an exacerbation of inflammation and infection in the airways. At first, it may be a minor inconvenience to the affected individual. Later in the course of the disease, as the baseline level of lung function deteriorates, these exacerbations can be life-threatening.

The mechanisms causing these acute exacerbations of asthmatic bronchitis are not completely understood. However, as the name "asthmatic bronchitis" suggests, there is much evidence that it, like asthma, is a disorder in which airways narrow due to constriction of the muscle cells surrounding the airways. In addition, inflammation of, and fluid accumulating in, the airway walls occur. In asthma, this process is completely reversible and is accelerated by appropriate therapy.

Treatment of chronic obstructive pulmonary disease. A patient with chronic obstructive pulmonary disease is using a hand-held nebulizer to administer a bronchodilator.

Much work over the past 40 years has gone into developing drugs that will cause the airways to open when constricted in such a fashion. These drugs, called bronchodilators, can be classified into several groups. The beta-adrenergic agonists are drugs that work through specific receptors in the airways. An example of such a drug is epinephrine, which is a naturally occurring hormone in man. When this agent binds to its receptor on a cell, for example, on a smooth muscle cell in the wall of a small bronchus, the result is a relaxation of the cell and, consequently, dilation of the airway. While effective in treating asthmatic bronchitis, the problem with beta-adrenergic agonists is that they also affect parts of the body other than the bronchial tree. Thus, their use can be associated with side effects, such as a rapid heart beat and muscle tremors.

Studies in the 1950s and 1960s demonstrated that there were actually two classes of beta-adrenergic receptors, the beta$_1$ receptors found in organs such as the heart and gastrointestinal tract and the beta$_2$ receptors found in the airways and organs such as the uterus. Using this fundamental knowledge, it soon became possible to design drugs relatively specific for the beta$_2$ receptors, hence avoiding many of the unpleasant side effects of the use of beta-adrenergic agents to treat asthmatic bronchitis.

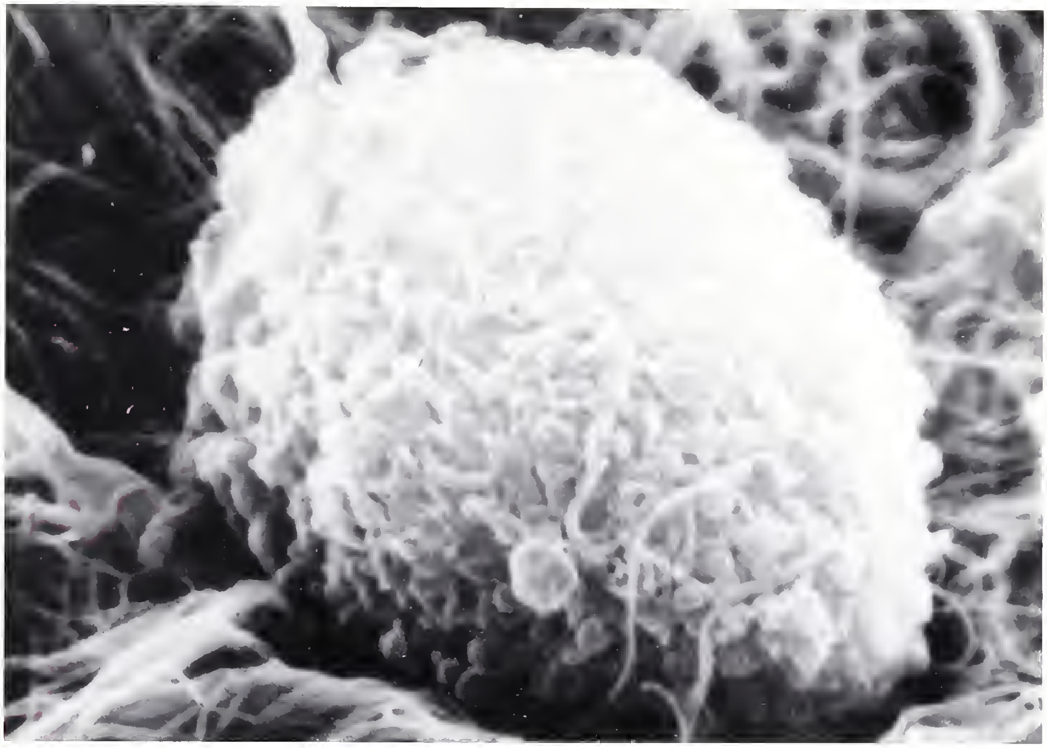
Another advance in the use of these agents has been in the development of aerosol forms of delivery. This approach targets the drug directly to the airways, reducing the dose necessary to be effective, and thus lessening the incidence of side effects of the drug. While beta-adrenergic agonists often are prescribed for patients with asthmatic bronchitis on a regular basis, they can be used intermittently in mild cases, usually by delivering the drug by aerosol directly to the constricted bronchi.

The methylxanthines are drugs known for many years and are closely related to caffeine, the stimulant in coffee and tea. Like the beta-adrenergic agents, the methylxanthines relax the smooth muscle cells in the walls of airways. But, they have many other effects, including stimulation of the clearance mechanisms in the bronchi. They increase the ability of the diaphragm, the large muscle separating the chest from the abdomen, to contract, thus helping patients with COPD to breathe more efficiently. One of the problems with the use of methylxanthines is that it is sometimes difficult to gauge the correct dose because there is a wide variation in how individuals metabolize the drug. This problem was, in the main, obviated by the development of methods to assay the level of this drug in the blood. As a result, physicians know the levels at which the drug is effective and the level above which side effects develop. In this context, methylxanthines can be used effectively; by monitoring the levels of these drugs, side effects such as gastrointestinal distress can be avoided. In recent years, many different forms of the methylxanthines have been developed with different absorption and excretion characteristics, thus helping the clinician to choose a preparation appropriate for each patient.

One of the new developments in this field has been the adaptation of the use of anticholinergic agents as bronchodilators. These drugs are cousins of atropine, or belladonna, and share with it the side effects of a rapid heart beat, urinary retention, and altered thinking. The development of modified versions of atropine, however, together with aerosol targeting to the airways, has led to the observation that anticholinergic agents can be useful for some patients with COPD.

Corticosteroids, drugs with the structure and properties similar to the natural body hormone cortisone, have been used in the treatment of asthmatic bronchitis since their introduction in the 1950s. These drugs can be very useful for some patients with this condition, but have serious side effects such as the induction of diabetes, weight gain, development of cataracts, and collapse of the vertebrae of the spine. However, by using aerosol preparations, the side effects of corticosteroids can be obviated, at least in part.

Although there have been major developments in the discovery and use of bronchodilators, the search for the "better mousetrap" still continues. Among the new approaches in this field is the development of calcium channel blockers, drugs widely used for treating certain cardiac problems but now recognized as also capable of bronchodilation. Other approaches include the use of prostaglandins, particularly those of the E series, which are naturally occurring substances capable of opening airways. Still under investigation, but showing promise, are classes of drugs that block the release of mediators that cause the airways to constrict. Other approaches involve drugs that prevent mediators from constricting the airways. These mediators, some released locally in the airways and others released elsewhere and brought to the lung, are thought to be the signals that cause bronchoconstriction. The concept behind the development of such drugs is



The alveolar macrophage. A scanning eletronmicroscope picture of the alveolar macrophage, the cell that defends the lower respiratory tract.

that if the release of these mediators can be prevented, or if the mediators can be halted from functioning, the result would be the prevention of airway closure.

Antimicrobial Therapy— The Suppression of Infection

Two of the three forms of COPD, bronchitis and asthmatic bronchitis, are characterized by intermittent infection of the airways with bacteria. At least in part, these infections occur because the damaged airways in COPD are unable to clear the bacteria that are normally inhaled in the air. In addition, as COPD progresses, the surface of the deranged airways becomes a haven for colonies of bacteria. In certain circumstances, for example, if the affected individual develops an influenza-like illness, these bacteria multiply. This causes the cells lining the airways to produce more mucus, and white blood cells come to the area to help fight the growing infection. The result is a thick phlegm that clogs the bronchial tree, further worsening the ability of the lung to exchange oxygen and carbon dioxide. The patient with COPD usually is alerted to a developing infection as the production of phlegm increases and it changes consistency and color, from thin and clear to thick and yellow or green.

Important investigations by NHLBI investigators in the 1950s and 1960s led to an understanding of how the airways normally defend against bacterial infection. In normal individuals, inhaled bacteria are removed from the airways by the mucociliary escalator, a continuous thin film of mucus constantly carried along the surface of the airways towards the mouth by the coordinate beating of cilia, thin hair-like projections on the surface of some of the cells lining the airways. A second line of defense is provided by macrophages, a mobile cell that is a tissue representative of a white blood cell. Macrophages are capable of engulfing or "phagocytozing" bacteria. Once inside the macrophage, the bacteria are destroyed.

In COPD, these defenses are less competent than in normal individuals. In part, this is due to the damage to the airways associated with COPD and, in part, due to environmental factors. For example, cigarette smoking causes the mucociliary escalator to work less efficiently and interferes with the ability of macrophages to clear the airways efficiently. Although a variety of species of bacteria often colonize the airways in COPD, the most common are *Streptococcus pneumoniae* and *Hemophilus influenzae*.

Oxygen therapy. In the late stages of chronic obstructive pulmonary disease, oxygen therapy provides the body with much-needed oxygen and significantly reduces mortality.

With the understanding that the normal defenses of the airways need help in bronchitis and asthmatic bronchitis, clinicians now aggressively treat developing bacterial infections in COPD patients with antimicrobials, classes of drugs referred to as antibiotics. Since the first antibiotics, sulfas and penicillin, were discovered, intensive study of the mechanisms of action of these drugs has led to a long list of drugs that can be used to treat the acute exacerbations of bronchial infection that are common in COPD. Among these drugs are ampicillin, a penicillin-like agent that has activity against a broader spectrum of bacteria than does penicillin, and a class of agents called the cephalosporins, drugs designed to circumvent the problem of bacteria that have become resistant to penicillin-like antibiotics. Other antimicrobials commonly used to treat infection associated with COPD include the combination drug trimethoprim and sulfamethoxazole, and tetracycline and its derivatives.

Although antibiotic therapy for COPD does not cure the disease, there is no question that it helps to prevent respiratory failure and improves the overall quality of life of affected individuals. Together with the maintenance of good nutrition and physical conditioning, and bronchodilators for those with asthmatic bronchitis, antibiotics play a central role in the therapy of COPD. Once used on an empiric basis only, the present understanding of lung defenses and the pathogenesis of COPD has led to the rational and effective use of these agents.

Oxygen Therapy—An Old "Drug" Leads to Decreased Mortality

A central consequence of COPD is the limitation of the ability of the lung to transfer oxygen from the air to the blood at the rate necessary for normal body functions. In the past, physicians often prescribed oxygen therapy for these individuals, in which purified oxygen was breathed through a mask or through nasal cannulas, thin tubes placed at the entrance to the nose. However, there were several problems with this empiric use of oxygen to treat COPD. First, sometimes patients with COPD worsened with oxygen therapy. Second, no one knew whether or not it really improved morbidity or mortality from the disease. Finally, the administration of oxygen was cumbersome, requiring large cylinders of gas, thus "tying" the patient to the site of the cylinder. Over the past 40 years, NHLBI investigators have solved all of these problems.

Basic studies concerning the mechanisms of the control of respiration have led to an understanding of what tells us to breathe, and why these processes go awry in COPD. Respiration is controlled by the brain in a section of the brain stem called the medulla. Although we can consciously override these controls, for example, by holding our breath for a limited period, these control processes are automatic. Like a thermostat that regulates the temperature in our homes when the furnace is on or off, the centers that regulate when we breathe monitor the levels of oxygen and carbon dioxide in the blood. When the oxygen is low or the carbon dioxide is too high, the system adjusts to breathe more often, more deeply, or both. In some cases of COPD, the carbon dioxide levels are chronically high, and the body adjusts to them by excreting acid in the urine, thus balancing the tendency of the high carbon dioxide levels to make the blood too acidic. When this adjustment is made, the mechanisms that respond to changes in blood carbon dioxide levels shut off and the drive for respiration becomes dependent on monitoring the levels of oxygen in the blood. However, when oxygen is administered to such an individual, the desired goal—an elevation of blood oxygen—can have disastrous consequences. If the carbon dioxide monitors have been switched off and, concurrently, the system "sees" normal levels of oxygen, the centers for respiration in the central nervous system see no reason to push the respiratory system. Consequently, respiration tends to slow, and the depth of each breath decreases. As a result, the administration of oxygen can have the opposite of the intended effect and the patient can worsen.

With a realization of this problem through basic research, physicians now know to monitor closely the levels of oxygen and carbon dioxide in the blood of patients with COPD to whom oxygen is going to be administered. They do this by taking a blood sample from an artery with a small needle and measuring the so-called arterial blood gases, which are the levels of oxygen, carbon dioxide, and acid in the blood coming from the heart and lungs. Once requiring cumbersome equipment and very time-consuming, the measurement of arterial blood gases is now done by automated, accurate, and rapid instruments found in all hospitals in the United States.

While administering oxygen to patients with low oxygen levels in their blood makes sense, it took two large-scale clinical trials to prove that chronic oxygen therapy was actually useful in treating advanced COPD. These trials, conducted in the United States and the United Kingdom and completed in the early 1980s, evaluated the effectiveness of long-term home oxygen therapy in patients with advanced

COPD. The study in the United States compared continuous oxygen therapy (oxygen administered at all times possible) with oxygen therapy for 12 hours per day, including the sleeping hours. Analysis of the data revealed that continuous oxygen use was better than the 12-hour "nocturnal" oxygen use, with the continuous therapy resulting in an average of more than 3 years of additional life for the affected individual. In the United Kingdom study, nocturnal oxygen therapy was compared with no therapy and the results were convincing. The survival of patients with COPD receiving nocturnal oxygen therapy was clearly better than for those not receiving oxygen. In addition to the survival advantages of oxygen therapy, COPD patients receiving oxygen therapy seem to have neuropsychological benefits, including improvement in objective measures of brain function such as IQ. Thus, oxygen, one of the oldest "drugs" known, clearly has beneficial effects when administered in a controlled fashion to patients with advanced COPD.

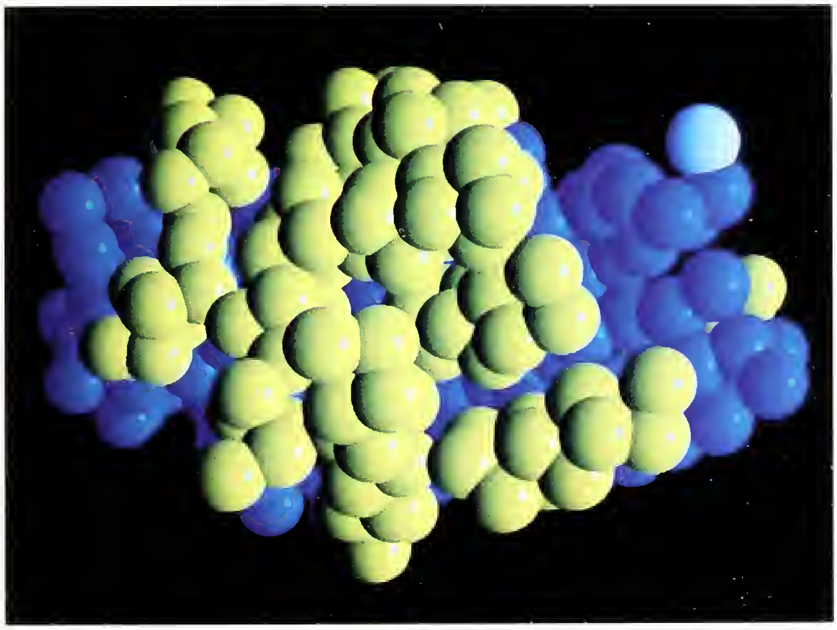
The mechanisms by which oxygen therapy is efficacious for advanced COPD have been studied intensively by NHLBI investigators. First, such therapy results in greater amounts of oxygen being transferred across the lung to the blood, thus permitting vital organs to receive more oxygen. Second, the decreased blood oxygen levels in advanced COPD are associated with constriction of the pulmonary arteries, the large blood vessels carrying blood from the right side of the heart to the lungs. Consequently, the pressure in the pulmonary arteries increases, a condition called pulmonary hypertension. On a chronic basis, pulmonary hypertension can cause the right side of the heart to fail, a condition called cor pulmonale. The available evidence suggests that chronic oxygen therapy will reduce pulmonary hypertension in these individuals and hence lessen the chance for the development of cor pulmonale.

While oxygen therapy is clearly useful in advanced COPD, oxygen is expensive and cumbersome to administer. Unlike a pill that can be carried anywhere and taken at will, continuous oxygen therapy requires a container for the gas and the tubing to transfer the gas from the container to the patient. With the evidence from large clinical trials that oxygen can be effective for some patients with COPD, attention has been placed on developing less expensive and less cumbersome methods of oxygen administration. Included among the advances in this field is the development of "oxygen concentrators," equipment that separates oxygen from nitrogen in air by the difference in size of the two molecules and concentrates the oxygen for administration to the patient. While less expensive than tanks of oxygen, this equipment is bulky and cannot be used easily by the ambulatory patient. An alternative approach has been the use of liquid oxygen. Advances in the technology of handling liquid gases have led to the development of compact devices weighing 10 pounds that can hold sufficient amounts of liquid oxygen to provide oxygen to the COPD patient for several hours of therapy.

Alpha-1-Antitrypsin Deficiency—An Experiment of Nature Leads to an Understanding of Emphysema

Among the three forms of COPD, emphysema is the only one that directly affects the alveoli where gases are actually exchanged. Although morphologists have long recognized that emphysema is associated with destruction of the walls of the air sacs, it took intensive study to understand why this occurs.

The walls of the air sacs are supported by a connective tissue matrix, the same kind of molecules that provide the structural support of skin, tendons, and all internal organs. Studies by NHLBI investigators have detailed the kinds of connective tissue present in the alveolar walls, how it is made, and what enzymes are capable of destroying it. The connective tissue is comprised mostly of type I collagen, a tough molecule that provides support but is not easily stretched. Also present is type III collagen, a more pliable matrix component, and fibronectin, an adhesive molecule that helps to bind cells to the matrix. These molecules are intertwined with proteoglycans, complex mixtures of proteins and sugars that together affect the function of the overall connective tissue matrix as well as modulate the flow of water and proteins passing through the alveolar walls. Critically, the alveolar walls also contain elastic fibers, molecules that have rubber-like properties that help the lung to exhale air during respiration.



Computer-generated three-dimensional model of the alpha-1-antitrypsin molecule. This protein protects the air sacs from injury by destructive enzymes carried by white blood cells. In the hereditary disease, alpha-1-antitrypsin deficiency, the reduced amounts of this molecule leave the lung vulnerable to attack and eventually to emphysema.

Using cell culture techniques, it has been demonstrated that these connective tissue components are made by cells within the alveolar walls, including fibroblasts, as well as by cells covering the air and blood surfaces of the alveoli. Studies with a variety of enzymes have shown that when the alveolar wall connective tissue is destroyed, it is very difficult for the cells to remake this matrix in a normal fashion. The elastic fibers are particularly important; if destroyed, the affected alveolus is effectively lost.

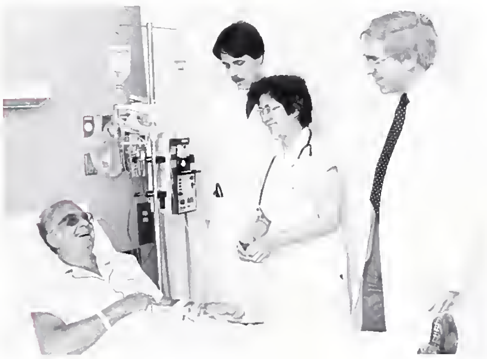
In reference to emphysema, the information relating to the composition of the alveolar wall matrix became particularly important with the discovery of an hereditary disease, alpha-1-antitrypsin deficiency, in the early 1960s. Alpha-1-antitrypsin is a protein produced by the liver and secreted into the blood where it diffuses through body organs to protect them from neutrophil elastase, a destructive enzyme carried by white blood cells that is capable of destroying the connective tissue backbone that serves as the structural support for tissues such as the walls of the air sacs. We inherit one alpha-1-antitrypsin gene from each parent. Most individuals have inherited the common M-type alpha-1-antitrypsin gene from both parents and have normal levels of alpha-1-antitrypsin in their blood and in tissues. In 1 to 2 percent of the population, however, one of the alpha-1-antitrypsin genes is the Z-type, differing from the most common normal alpha-1-antitrypsin gene by two amino acids. If the Z gene is inherited from both parents, as occurs in 20,000 to 40,000 individuals in the United States, the alpha-1-antitrypsin levels in the body are about 15 percent of normal. Because alpha-1-antitrypsin provides the major defense of the body against neutrophil elastase, sites at which neutrophils accumulate cannot deal with the increased burden of elastase in the local milieu. As a consequence, the neutrophil elastase has unimpeded ability to destroy the elastic fibers as well as other connective tissue components of the alveolar walls.

That is exactly what happens in the lung in alpha-1-antitrypsin deficiency. The lung is the only organ in the body that chronically must contend with a burden of neutrophils. While this is a low burden that is easily handled by the protective screen of alpha-1-antitrypsin in normal individuals, in those who have inherited the Z gene from both parents and hence have alpha-1-antitrypsin deficiency, this low burden of neutrophils releases sufficient amounts of elastase to slowly destroy the alveolar walls. In those individuals with alpha-1-antitrypsin deficiency who smoke cigarettes, the process is accelerated, so much so that they have a 10- to 20-year shorter lifespan than alpha-1-antitrypsin deficient individuals who do not smoke. In normal adults, there are approximately 300 million alveoli, far more than are needed by most of us in our everyday activities. Consequently, many alveoli—probably 20 to 30 percent for active individuals and perhaps more for those who are sedentary—can be destroyed before the affected individual detects a problem and seeks medical attention. Thus, alpha-1-antitrypsin deficiency usually is not diagnosed until the individual is in his or her late 20s or early 30s, and sometimes later.

Of all cases of COPD in the United States, alpha-1-antitrypsin deficiency represents a relatively small proportion, estimated at about 2 percent of the total. Furthermore, unlike most cases of COPD, individuals with alpha-1-antitrypsin deficiency usually have emphysema without concomitant elements of bronchitis or asthmatic bronchitis. However, although alpha-1-antitrypsin deficiency explains the pathogenesis of what appears to be only a small percentage of the total cases of emphysema, in fact, it has become the model for understanding the development of emphysema in general.

In this regard, while individuals with normal levels of alpha-1-antitrypsin should have adequate amounts of alpha-1-antitrypsin in the lungs to protect against destruction by neutrophil elastase, studies by NHLBI investigators have shown that this defensive molecule has an "Achilles' heel" that permits it to be inactivated, leaving the lung relatively defenseless against neutrophil elastase. Alpha-1-antitrypsin inhibits elastase at a specific site, called the active site, in the alpha-1-antitrypsin molecule. Centered in this active site is the amino acid methionine, a common amino acid that is very susceptible to oxidation, a process that removes electrons, causing the methionine to change its form. When this occurs, for example, in the presence of cigarette smoke or oxidants released from inflammatory cells activated by cigarette smoke, the alpha-1-antitrypsin molecule no longer can act as an effective inhibitor of elastase. This concept, while still being investigated actively, has helped enormously in explaining why cigarette smoking is associated with the development of emphysema.

For alpha-1-antitrypsin deficiency, an understanding of the pathogenesis of the disease has led to major strides in the development of what should be effective therapy for the prevention of further destruction of the alveoli. By purifying alpha-1-antitrypsin from the serum of normal donors, NHLBI investigators have demonstrated that weekly intravenous infusions of alpha-1-antitrypsin to alpha-1-antitrypsin deficient individuals can maintain plasma and lung alpha-1-antitrypsin levels that should be sufficient to protect the alveoli from the burden of neutrophil elastase to which they are exposed. Purified alpha-1-antitrypsin prepared by commercial sources is now being evaluated. Furthermore, using using methods of recombinant DNA technology, human alpha-1-antitrypsin has been manufactured in microorganisms. Such molecules have been shown to be effective as inhibitors of neutrophil elastase in the test tube, and studies are in process to evaluate their potential use in humans. The prospect of using recombinant DNA technology to produce alpha-1-antitrypsin has several advantages. First, since the microorganisms can be grown in vast amounts, the supplies would be unlimited. Second, the recombinant products would be free of potential viral contaminants inherent in any blood-derived product. Finally, using the recombinant technology, it is possible to modify the alpha-1-antitrypsin molecule such that the methionine at the active site is replaced with valine, another amino acid. This modified form of alpha-1-antitrypsin not only inhibits neutrophil elastase effectively, but importantly, is not susceptible to oxidation. Thus, for the subgroup of COPD patients with alpha-1-antitrypsin deficiency, the prospects are excellent for one or more therapeutic agents that should prevent further lung destruction.



Administration of alpha-1-antitrypsin to an individual with the hereditary disorder, alpha-1-antitrypsin deficiency. By augmenting the levels of alpha-1-antitrypsin in the blood and lung, the air sacs are protected from progressive destruction.

Summary

A common lung disease that disables millions of American people, COPD has been under intensive scrutiny by NHLBI-supported investigators. Immense strides have been made.

The major causative agent, the cigarette, has been identified. Sophisticated physiologic techniques can detect COPD in its early stages. Drugs are available that improve the quality of life of affected individuals and, in some cases, extend the lifespan of those with severe disease. For the small subgroup of individuals with the hereditary disorder, alpha-1-antitrypsin deficiency, the molecular mechanisms of the pathogenesis of COPD are now defined and therapies have been devised that should prevent the progressive nature of the disease.

The past 40 years of progress give reason to believe that the enigma of COPD has been deciphered. All indications suggest that progress in this field, together with the avoidance of cigarette smoking, will result in the reduction and eventual elimination of this disabling disease.

Ronald G. Crystal, M.D.

(Left) A model of a hemoglobin molecule showing points of mutation.

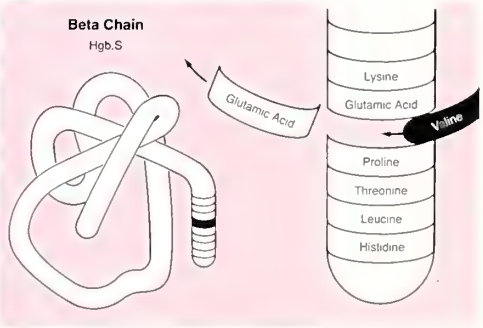
Hemoglobinopathies: Achievements In Molecular Biology

Dr. James Herrick described "peculiar elongated and sickle-shaped red blood corpuscles" in a Black West Indian student in 1910.

For many years, hematologists had been intrigued by a group of hemolytic anemias of hereditary origin that were defined on the basis of their varying clinical and morphologic findings. Among the major examples are sickle cell anemia, described by Dr. James Herrick in 1910, and Cooley's anemia, described in 1925. However, little was understood about the etiology of these disorders until abnormalities of hemoglobin, the red pigment of blood responsible for carrying oxygen to the tissues, were identified by Linus Pauling and Harvey Itano in 1948. These critical observations took place about the time that the National Heart Institute (later to be called the National Heart, Lung, and Blood Institute) was being established as the second of the categorical Institutes of the NIH.

Evolution in Knowledge of Hemoglobin

In 1948, hemoglobin already had been the subject of extensive study for over 100 years from both the structural and physiological points of view. Much was known about its composition, physical nature, and chemical characteristics. In addition, it had been recognized for almost as long a period that infants had a distinctive form of hemoglobin, called fetal hemoglobin (HbF), that differed in important physiological ways from its normal adult counterpart. The observations of Pauling and Itano, indicating a structural abnormality in the hemoglobin found within the red cells of a patient with sickle cell anemia, represented a discovery of the most profound importance. In 1958, Ingram demonstrated that hemoglobin S (HbS) resulted from the substitution of a single amino acid six units down from the N-terminus of one of the chains (beta chain) of the hemoglobin. Subsequent use of the methods employed in this discovery led to not only the recognition of many other abnormal hemoglobins but also an understanding of the genetic



(Right) Beta chain with single amino acid substitution, valine for glutamic acid.

code, the mechanism of initiation of protein synthesis, and today's intoxicating progress in molecular biology and genetic engineering.

By the time that NHLBI had instituted its hematology program during the latter part of the 1960s, much work already had been carried out to define the nature of the structural characteristics of normal adult (HbA) and fetal hemoglobins, as well as the characteristics of an increasing number of abnormal hemoglobins, some intimately connected with hemolytic anemias prevalent throughout the world. Included in this group were HbS, hemoglobin C (HbC), hemoglobin D (HbD), and hemoglobin E (HbE) in various forms and combinations of sickle cell disease and thalassemia, as well as many other variants of hemoglobin, some of which had little or no clinical significance.

Support for many of the seminal studies related to the abnormal hemoglobins was provided throughout the Institutes of the NIH between the 1940s and the mid-1960s, although the National Institute of Arthritis and Metabolic Diseases (NIAMD) and the National Institute of General Medical Sciences served as focal points for such work during those exciting days of exploration. The NHLBI began its involvement in this area of research when it instituted a blood diseases and resources program, initially as a branch of NHLI and subsequently as a formal Division of NHLBI. Thus it is only during the past 20 years of this process that NHLBI-supported scientists, both intramurally and extramurally, have been intimately and creatively involved in this field of scientific inquiry.

Hemoglobin, the heme-protein complex from which the red corpuscles derive their function as oxygen carriers, consists of four iron-bearing heme groups and two pairs of dissimilar polypeptide (globin) chains. The amino acid sequences and rates of synthesis of the globin chains in the red cell precursors are controlled by structural and regulatory gene sequences in the DNA-RNA code. Excluding transient embryonic variants, four kinds of globin chains, designated alpha, beta, gamma, and delta, are synthesized. The genes coding for alpha chains are active from an early stage of gestation through adult life so that one of the two pairs of globin chains is always alpha. The expression of the genes coding for the complementary pair changes during the transition from fetal to extrauterine life, but in normal individuals, the synthesis of alpha and nonalpha chains is always balanced. Most of the postnatally synthesized hemoglobin (HbA) consists of alpha- and beta-chain pairs.

Sickle cell anemia and Cooley's anemia (thalassemia major, beta-thalassemia*) are prototypes of a large and diverse group of disorders arising from mutations of globin

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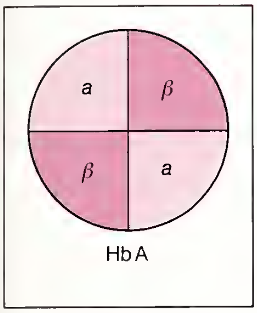
**The term "thalassemia" (from the Greek* thalassa, *the sea, and* haima, *blood), coined in 1932 in the belief that Cooley's anemia was peculiar to persons of Mediterranean descent, has displaced the eponym in scientific usage for the sake of convenience. It can be hyphenated to discriminate between alpha- and beta-thalassemias, and it lends itself to denoting hybrid conditions such as sickle cell-thalassemia and HbE-thalassemia. Collectively, the term "thalassemias" describes a multiplicity of disorders of which Cooley's anemia was only the first to be recognized. The geographic distribution of thalassemias extends from the Mediterranean basin to the Far East. In the United States, beta-thalassemia genes have a significant incidence in ethnic groups of Italian, Greek, and Oriental descent. They are also found—though far less often than the sickling genes—in black Americans. On the other hand, alpha-thalassemia occurs widely not only in those of Asian and Mediterranean origin but also in black Americans.*

genes and resulting in either the production of structurally abnormal hemoglobins or the impairment and imbalance of the synthesis of normal hemoglobin. Homozygotes (persons who have inherited the same mutant gene from both parents) may die in childhood or suffer from lifelong, often severely disabling disease that requires frequent blood transfusions and treatment for complications, notably infections and damage to vital organs. Heterozygotes (carriers of a single abnormal gene) exhibit an asymptomatic but readily detectable trait, e.g., sickle trait or "Cooley trait" (thalassemia minor). Tests for these traits are essential for genetic counseling and for population surveys.



Simplified model of Hemoglobin A.

Four decades ago, the molecular basis of these disorders (now known collectively as hemoglobinopathies) was only suspected, and even their genetic aspects were not fully understood. Our present knowledge reflects some of the major achievements of modern molecular biology: elucidation of the molecular structure of hemoglobin, precise characterization of its normal and pathological variants, evolution of the concept of alpha- and beta-thalassemias, and discovery of the basic genetic mechanisms by which the synthesis of globins (or any other proteins) is controlled at the cellular and molecular levels.

A National Effort to Combat Hemoglobinopathies

In 1971, President Nixon sent a message to Congress in which sickle cell anemia was targeted as a major health problem in this country. Recognizing the magnitude of the problems posed by sickle cell disease and the thalassemias, Congress enacted two laws in 1972, The National Sickle Cell Anemia Control Act (P.L. 92-294) and The National Cooley's Anemia Control Act (P.L. 92-414), followed the same year by the comprehensive National Heart, Blood Vessel, Lung, and Blood Act (P.L. 92-423). In response, NHLI designated sickle cell anemia as a National Program Area and established a Sickle Cell Disease Branch in the newly created Division of Blood Diseases and Resources (DBDR) charged with promoting research, training, education, and related activities. Comparable responsibilities for Cooley's anemia and thalassemia in general were assumed by the Blood Diseases Branch of DBDR, with additional support from the Division of Kidney, Urologic, and Hematologic Diseases of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK).

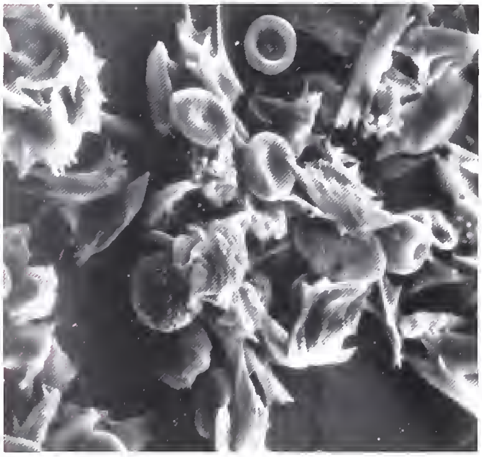
Since the early seventies, therefore, the Institute has played a major role in developing the research base for sickle cell disease and Cooley's anemia. At the time of the President's message to Congress in 1971, NIH research activities that were related to sickle cell disease amounted to approximately $1 million distributed among several Institutes. NHLI took the lead for this Federal initiative, organized a national program of research and services, and provided the catalyst for research support that for the current year is in excess of $25 million.

In a similar vein, the Institute has provided a major focus for thalassemia-related research, sharing some aspects of this area of responsibility as noted above with NIAMD (subsequently NIADDK and currently NIDDK). Current support for Cooley's anemia research at the NHLBI is over $6 million.

Sickle Cell Disease — Spectrum of Research

The modern era of research in sickle cell disease followed passage of the National Sickle Cell Anemia Control Act of 1972 and the assignment of responsibilities for this program to NHLI. The stimulus provided by this effort aroused the interest of a large number of biochemists, cell biologists, physical chemists, and molecular and structural biologists.

The accomplishments since 1972 can be divided broadly into basic molecular and cellular studies, clinical studies, and therapeutic trials. In addition, a significant feature of what has been achieved is the interaction among experts in each of these fields. The NHLBI program from the outset sought to improve communication among basic and clinical scientists, educators, practicing physicians, and the community at large. Much has been accomplished in fostering those interactions, although much remains to be done. This spectrum of research is described in the following sections.

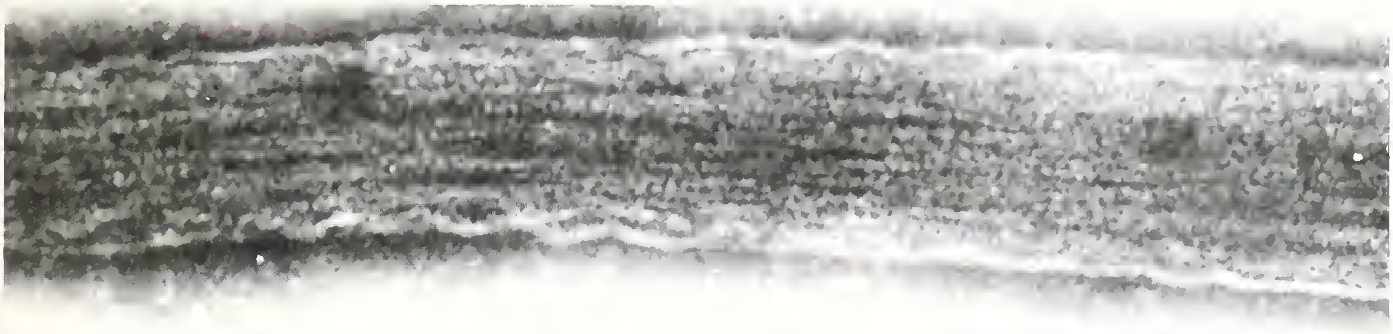


Sickled cells magnified by a scanning electron microscope.

Basic Molecular and Cellular Studies

The recognition that gelling of the abnormal HbS leads to sickling of the red cell and that partial inhibition of gelation could reduce clinical severity has led to an enormous effort to understand the gelation process in detail and to relate the results of these studies to the pathophysiology and therapy of sickle cell disease. From such studies emerged the picture that a gel of HbS is composed of long polymers, which have the appearance of fibers, together with hemoglobin molecules free in solution. Two major advances in structural studies of individual fibers have been made. One has been the demonstration by electron microscopy and image reconstruction techniques that the fiber consists of 14 intertwined helical strands. Bundles of these fibers are responsible for sickling, i.e., deformation of the red cell. The second has been the determination by x-ray crystallography that the crystal is composed of double strands in which one beta 6 valine in each tetramer is involved in an intermolecular contact between molecules on adjacent strands. The double strand of the crystal is a basic structural element of the fiber so that the fiber can be constructed from seven helically twisted double strands. These structural studies have guided the development of agents designed to inhibit gelation by altering the conformation of the HbS molecule or by directly interfering with the formation of bonds between molecules in the fiber by blocking the contact sites.

Kinetic studies of gelation not only led to a mechanism for the formation of fibers but also have played a central role in elucidating the pathophysiology of sickle cell disease and suggesting new therapies.



Fiber from Hemoglobin S, bundles of which are responsible for sickling.

These studies showed that gelation is preceded by a marked delay period that is extraordinarily sensitive to ambient conditions. The importance of the delay time to the occurrence of sickling is that it allows most cells to escape from the microcirculation and be reoxygenated in the lungs before any significant polymerization has occurred. In the absence of a delay period, over 90 percent of cells would contain polymer and be sickled. In the case of sickle cell trait, the delay times are of sufficient length that, with the possible exception of the kidney, all cells return to the lungs before any polymerization of the hemoglobin has occurred, accounting for the lack of any clinical signs of disease. In sickle cell anemia, this delay time becomes the critical factor that leads to clinical disease. Differences between genetically defined syndromes therefore can be explained by differences in intracellular gelation rates. There is, however, a wide spectrum of clinical severity within each of these disorders, and this heterogeneity is currently a subject of intense research. One possibility is that differences in the degree of adherence of red cells to the vascular endothelium are responsible. Adherence has the effect of slowing blood flow and thus increasing the transit time through the microcirculation and allowing more cells to sickle.

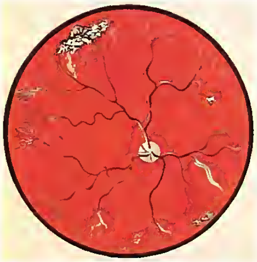
The overall impact of studies on gelation has been to introduce quantitative techniques and concepts of physical chemistry into the understanding of sickle cell disease. As a result, a comprehensive description of gelation and its relation to the pathophysiology and therapy of the disease is now available. These studies may be viewed as among the first successful applications of physical chemistry to a human disease.

Clinical Research

Among the NHLBI's major contributions to the study of sickle cell disease is the establishment of a number of Comprehensive Sickle Cell Centers as major program components. Designed to bridge the gap between scientific investigation and the practical application of this knowledge, centers were established (starting in 1973) in areas with large at-risk populations for sickle cell disease. This concept encouraged community involvement and the integration of health services into a research environment as part of a unique program emphasizing prevention, education, and control activities. Center grants have continued as the mainstay of the National

Sickle Cell Disease Program, and current legislation requires that the NHLBI maintain funding for a minimum of 10 such Centers.

A number of major advances in clinical studies in sickle cell disease, including prenatal diagnosis and a comprehensive description of the natural history, have emerged. Early attempts at prenatal diagnosis were based on the identification of the hemoglobin types found within the fetal red cells. The discovery of linked polymorphisms, readily recognizable changes in DNA that were inherited with the sickle cell mutation, revolutionized the field. The subsequent discovery of restriction endonucleases, enzymes capable of cutting genes at specific locations, that recognized the exact site of mutation proved even more important in that it permitted accurate diagnosis of sickle cell anemia from fetal cells in amniotic fluid (amniocentesis being a much safer procedure than sampling of fetal blood). The recent development of chorionic villus sampling and in vitro methods for DNA amplification may make earlier diagnosis a completely practical technique.



Sickle cell retinopathy

Although the first patient with sickle cell anemia was described over 75 years ago, a clear understanding of the disease is just beginning to emerge from the large NHLBI-sponsored study of the clinical course of a broad sample of patients. The study group represents the largest sickle cell disease population ever enrolled in a clinical research protocol (over 4,000 patients) and includes approximately 600 newborns. Growth and development, assessment of spleen function, major organ complications, psychosocial dysfunction, and longevity are among the topics investigated. Other studies have focused on the mechanisms of fulminant infection in young children, particularly as complement activation is impaired and splenic function is compromised or absent. Recognition of the hazard of infection with pneumococci was a strong stimulus to the development of a pneumococcal vaccine and, subsequently, to the recent demonstration that penicillin prophylaxis has a significant effect in decreasing mortality. Detailed studies of retinal pathology related to hemoglobinopathies led to the development of internationally recognized guidelines for the classification of ocular sequelae of these disorders. These studies in turn, have been instrumental in devising more effective therapy for the ocular pathology seen in sickle cell disease.

The onset of chronic organ damage appears earlier than previously expected. Gallstones can occur in children as young as 5 years of age, and aseptic necrosis of bones may be apparent as early as 3 years of age. Strokes are an important cause of death and disability in children, and transfusion regimens have emerged as an important factor in preventing them. Iron overload is an inevitable consequence of such transfusion; more effective use of the available chelating agent, deferoxamine, has been advanced, and an active search is underway for an orally effective agent. Information on the age of onset, rate of progression, and outcome of various complications, coupled with increasing knowledge of risk factors and the psychosocial implications of sickle cell disease, provide the foundation for improving the management and quality of life for these patients and their families.

The physician is examining this baby for possible sickle cell disease in one of the NHLBI Comprehensive Sickle Cell Centers.

An early focus of the NHLBI program was on emergency room management of sickle cell crises and the development of treatment protocols that would ensure adequate care for all patients. A consensus of experts in the field led to publication of "Management and Therapy of Sickle Cell Disease," an NHLBI booklet outlining the basics of care for common medical and surgical problems. Psychosocial dysfunction, a problem for many patients, had been recognized, and attempts have been made to improve its diagnosis and management. Nutritional disorders also have been suggested as part of the disease, at least in children, and studies directed at this problem are being encouraged.

A major contribution to the management of sickle cell patients has come from advances in education and counseling. The technology for population screening was well advanced before techniques for education and counseling were developed. One of the first goals for the Sickle Cell Centers and Screening Clinics, initially established in 1972, was the development, utilization, and assessment of educational programs. That objective has been met more successfully than expected because knowledge of the disease and its inheritance has spread far beyond the Centers. The development of counselors and counseling techniques has been less successful. Many states have instituted newborn screening programs for infants with clinically significant disease, but there are usually insufficient resources to provide follow-up and counseling to the mothers of infants with sickle cell trait detected during the screening process.

Education of medical personnel has been enhanced so that the disease has become less of an obscure curiosity. In urban centers, there are now adequate numbers of trained doctors and nurses to care for the newborns identified in screening programs. This generalization is not true in many rural areas but is being addressed through formal and informal training programs.

Search for a Treatment

Much of sickle cell disease research has been directed toward the development of a specific therapy. As noted above, the first effective form of therapy was transfusion, and it remains the major avenue of treatment for most patients. Iron overload, alloimmunization, hypertransfusion regimens, improved blood preservation, and techniques for transfusion have been addressed by NHLBI-sponsored projects.

Among the most important therapeutic investigations were the controlled cooperative trials of urea and alkali instituted in 1974. Although both agents were shown to be no more effective than placebo, the techniques developed during these trials for the description and assessment of pain and the widespread recognition that concurrent controls were absolutely essential for the evaluation of therapy for this subjective disorder provided models for subsequent trials in this area in the United States. In addition, many of the professional relationships forged during those trials led directly to a cooperative study of the clinical course of the disease.

Although urea was shown to be ineffective in vivo, its in vitro effects led to the consideration of cyanate as a form of treatment. Early studies indicated that it had a potent antisickling effect, which was later shown in kinetic studies to result both from an antigelation effect and from increasing the oxygen affinity of carbamylated sickle cells. Although early clinical trials demonstrated some promise, a later controlled trial showed no benefit at the dosage of cyanate used. Higher doses of cyanate were precluded by the description of neurotoxicity in several patients, but since the drug had such promise, an extracorporeal carbamylator was designed based on the premise that treatment would be safe if cyanate reacted only with red cells; virtually no unreacted drug was returned to the patient. By combining an extracorporeal reactor coil, a hemodialysis device, and appropriate sampling and warning systems, an effective carbamylator was built. In the end, its use did not prove to be effective; however, the availability of this instrumentation may be useful for other therapeutic approaches in the future.

Extracorporeal hemodialysis device once used in the treatment of sickle cell disease.

Current research on therapy is based on strategies that have been suggested from gelation studies. The correlations between gelation and clinical severity among the different genetically distinct sickling disorders indicate that it will not be necessary to inhibit gelation completely but that a therapeutic effect should result from increasing the delay time long enough to allow more cells to escape the microcirculation and be reoxygenated in the lungs before gelation begins. This correlation has served as a guide for determining the extent of inhibition necessary to obtain a specific therapeutic effect. The results suggest that there are at least three viable therapeutic strategies: blocking intermolecular contact formation in the polymer, decreasing the total hemoglobin concentration, and promoting fetal hemoglobin production. Contact formation can be interfered with either by competitive binding of an inhibitor at a contact site on the molecular surface or by changing the conformation of the molecule in the region of the intermolecular contact. A number of promising agents have been developed based on the results of gelation studies and structural results from x-ray crystallography. The total hemoglobin concentration can be decreased either by increasing the red cell volume or possibly by decreasing heme biosynthesis as in iron deficiency anemia. Because of the enormous sensitivity of the delay time to intracellular concentration of hemoglobin, small decreases are predicted to have a significant therapeutic effect. A preliminary clinical trial, in which the electrolyte balance was changed so that extra water entered the erythrocytes in patients treated with desmopressin acetate, gave promising results, supporting the idea that producing swelling of red cells could result in a clinical benefit.

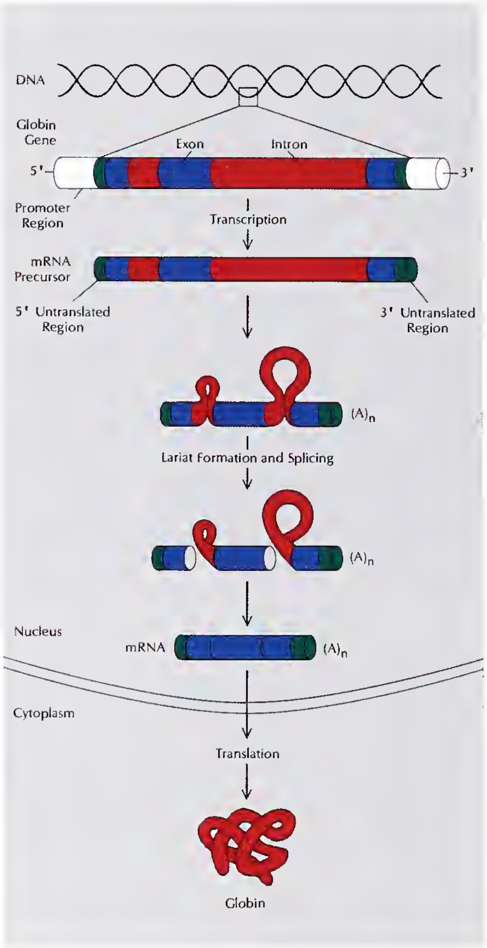
The strong inhibitory effect of HbF on gelation has pointed to increasing F cell production as a means of therapy. This work began with a systematic study of factors governing fetal hemoglobin synthesis in baboons. Methylation of DNA is believed to be a signal for the repression of fetal protein synthesis. This information led to the use of 5-azacytidine, a known hypomethylating agent, which resulted in impressive increases in HbF synthesis in anemic baboons. Similar effects could be achieved in patients, but clinical efficacy could not be assessed because of potential carcinogenicity of the drug. In attempts to clarify azacytidine's mode of action, it was shown that hypomethylation probably was not involved, and suggestions were raised that efficacy might be related to the drug's cytostatic properties. If that were true, other cytostatic drugs also should be effective in stimulating fetal hemoglobin synthesis—studies in animals confirmed this proposal. Clinical trials showed hydroxyurea stimulated fetal hemoglobin production, and a small cooperative study of the drug to determine optimum dosage is being organized. Hydroxyurea does not appear to be carcinogenic, but the trial is limited to a few severely affected patients until patient safety is assured.

Finally, it should be pointed out that marrow transplantation and genetic engineering may play a significant role in the treatment of sickle cell disease in the future. If sickle red cell precursors could be replaced by normal ones, or normal globin genes could be inserted into the patient's genome, the disease could be eliminated in treated persons. Neither technique is ready for application in sickle cell disease, but basic investigations of erythropoiesis and gene cloning and insertion are of considerable interest to the NHLBI. As with studies of more conventional antisickling agents, a broad base of related knowledge is an absolute prerequisite for the development of effective means of treatment.

Cooley's Anemia — A Model for Molecular Disease

Following Dr. Cooley's description of the disease that bears his name, major aspects of the genetic, clinical, and pathological features of this hemolytic anemia became clear. Nevertheless, a true understanding of what was at the root of the pathophysiology emerged only as a result of Pauling's discovery in 1948 of an abnormal hemoglobin in sickle cell anemia. Although sickle cell anemia differs from thalassemia in that the defect in the former is in the molecular structure of the hemoglobin molecule rather than in its amount, the concept that alterations in the structure of a protein or in the regulation of its synthesis could be involved in the genesis of pathology stimulated others to begin thinking about thalassemia as a molecular disease.

Cooley's anemia and other disorders of hemoglobin structure and synthesis have been studied in great depth. These diseases lend themselves to fundamental investigation at the molecular level for three reasons. First, they are diseases of red blood cells, and blood can be obtained easily from a patient. Second, red cells make large amounts of one protein, hemoglobin, while most other cells synthesize significant quantities of many different proteins. Thus problems in the production of hemoglobin tend not to be masked by the synthesis of other proteins. Third, the precursor red blood cell matures into a reticulocyte, a cell that has lost its nucleus and is essentially a "bag" of protein-synthesizing machinery making one primary product, hemoglobin.



Globin gene DNA is transcribed into precursor messenger RNA in the cell nucleus. After translation of mRNA into globin in cytoplasm, a heme prosthetic group is added to each globin chain, and a tetramer of alpha-like and beta-like globin chains forms mature hemoglobin.

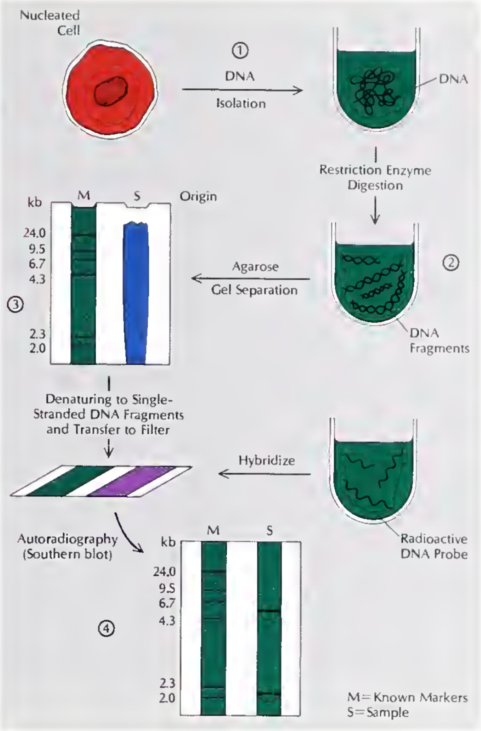
The relatively small group of patients with Cooley's anemia served as a valuable resource to investigators trying to understand human biology. Patient participation in such studies led to major advances in our understanding of the control of gene expression in humans. In turn, the fundamental knowledge that emerged became critical to the study of a multitude of other disorders of abnormal gene function, including diabetes, cancer, and cardiovascular disease.

Basic Molecular and Cellular Studies

Both intramural and extramural scientists have played central roles in research related to Cooley's anemia and hemoglobinopathies. The origins of such studies can be traced clearly to the elucidation of the genetic code, which provided the first clues to the structure and organization of genes. A developing interest in protein synthesis and its application to the study of patients with Cooley's anemia followed. Cell-free extracts for studying the synthesis of hemoglobins and other proteins were developed, and initiation factors were identified, characterized, and purified.

These studies laid the groundwork for the isolation of messenger RNA (mRNA), the chemical entity responsible for carrying the genetic code from the DNA of the gene to the actual synthesizing machinery. Further characterization of these messengers clearly implicated mRNA as being defective in thalassemia patients. As the technology

Prenatal diagnosis of hemoglobinopathies has been achieved with techniques of molecular biology. In Southern blotting analysis, described here, DNA is isolated from peripheral white blood cells or other tissue and analyzed to detect genetic abnormalities.



to approach these aspects of genetic disease developed, such findings directed the attention of investigators to mRNA metabolism and gene structure.

In 1972, the hemoglobin switching problem began to be a topic of interest. In the sheep, it was recognized that the switch from HbA to HbC represented a switch in "globin gene" expression. Further study of the sheep switching model led to an understanding of the initial phases of molecular switching. This information was applied, using the then-novel techniques of clonal hematopoietic cultures, to reproduce the switch in vitro. These efforts culminated in the demonstration that immature red cells could be "committed," by brief differentiation periods, to a specific pattern of globin gene expression. These results formed the basis for the conclusion that modulation of globin gene expression occurs within the context of hematopoietic stem and progenitor cell differentiation. Another major development was the use of somatic cell hybrids to assign the globin genes to individual chromosomes by techniques of molecular analysis and studies of globin gene expression.

Beginning in the mid-1970s, studies of hemoglobin switching were stimulated further by an NHLBI initiative. The grants funded under that program soon led to a major effort on this fundamental biological problem. These efforts developed and refined in vitro assays for the study of hemoglobin switching. The early observations from the sheep system were confirmed and extended greatly, and the fundamental message that switching is modulated during stem and progenitor cell differentiation has been confirmed.

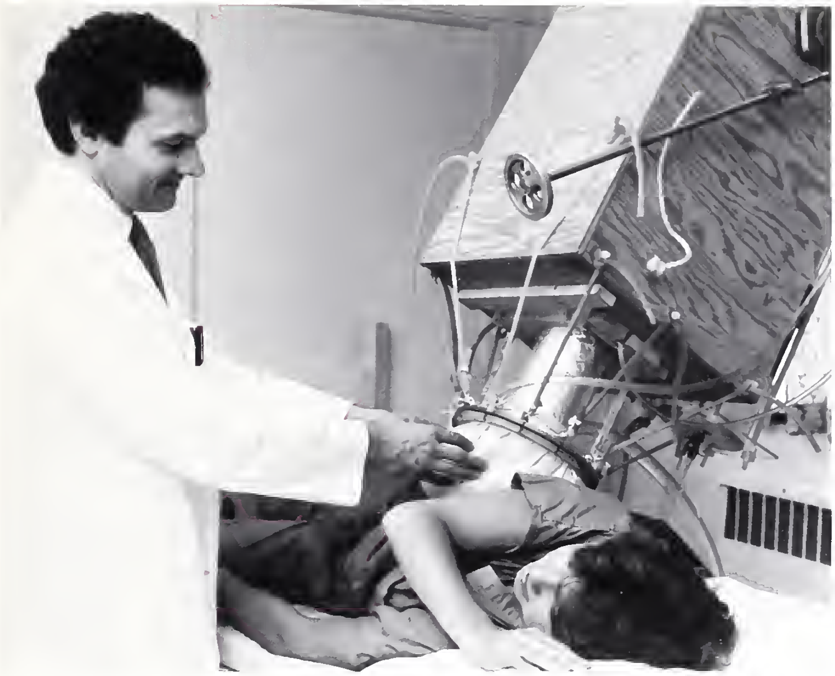
Study of the switch problem led to the use of drugs in 1982 to enhance fetal hemoglobin synthesis. Although progress in this area has been slow during the past 5 years, this effort provides a major opportunity for the manipulation of gene expression for therapeutic benefit. Just on the horizon is the ability to use hematopoietic growth factors (e.g., erythropoietin and/or interleukin-3), alone or in combination with the various drugs that have already been employed, to stimulate HbF synthesis. The ability to clone gene sequences that are complementary to the DNA code is a major accomplishment that paved the way for complete elucidation of the genetics of the thalassemia syndromes. Refinement of the molecular cloning strategies combined with haplotype analysis using restriction enzyme polymorphisms allowed investigators to define point mutations that alter globin gene expression. This methodology, along with the ability to assay gene function quickly in tissue culture cells, led to a complete elucidation of these defects. Other contributions led to improved prenatal diagnosis and to the molecular characterization of the thalassemias.

In recent years, a mouse model for Cooley's anemia has been developed, and these thalassemic mice are being used to examine methods for the selective introduction of genes into bone marrow cells using strategies that may be clinically applicable. As noted earlier, a primate model is available to investigate the potential of various drugs for manipulating fetal hemoglobin synthesis. The availability of such models allows drugs to be tested in combination with newly available substances that regulate the formation and development of blood cells. These regulatory growth factors have been produced by biotechnology firms using recombinant DNA techniques and are almost ready for clinical trials.

Future steps in combating Cooley's anemia will include gene therapy, the insertion of normal beta-globin genes into the blood-producing bone marrow cells of patients. For the moment, however, interest in gene therapy has shifted to investigations of single-gene enzyme deficiency diseases, such as severe combined immunodeficiency disease, which pose less complex problems of regulation. Nevertheless, the NHLBI continues to promote this area of research and currently supports a program entitled "Regulation of the Cloned Human Beta-Globin Gene," in which scientists are investigating a variety of recombinant DNA techniques to transfer and regulate normal human beta-globin genes in bone marrow cells in culture and in live animals. They are optimistic that, on completion of this program, it may be possible to formulate human gene therapy protocols for Cooley's anemia.

Clinical Research and Therapy

Inasmuch as genetic intervention at the molecular level is a possibility only for the distant future, what is required urgently in the meantime are improved therapies for the clinical management of patients with Cooley's anemia. Blood transfusions have been utilized for alleviating the severe anemia, but the repeated transfusion of red cells introduced massive amounts of iron into the body, which was deposited in vital organs. As a result of the deleterious effects of iron, death invariably occurred by the teenage years or early twenties. An iron chelating (binding) drug, deferoxamine, was available to treat acute iron poisoning, and it was suggested that this drug might be useful for treating chronic iron overload. The NHLBI initiated a program entitled "Improved Methods for the Clinical Management of Thalassemia" to encourage investigators to study the efficacy of deferoxamine in the treatment of chronic iron overload and to develop better transfusion techniques to improve the clinical status of patients. This effort was followed by a second effort several years later on the same subject. Implementation of these programs was made possible when the sole supplier of deferoxamine agreed to provide this extremely costly drug free to NHLBI grantees and intramural scientists engaged in these studies.



Technician using a superconducting quantum interference device (SQUID), the prototype of a newly developed device to measure, noninvasively, the amount of iron stored in the liver.

A Cooley's anemia patient receiving red blood cell transfusion.

Deferoxamine given subcutaneously to patients resulted in a negative iron balance. There was, however, the need to determine whether deferoxamine would prevent cardiac damage in children who began therapy before significant iron buildup had occurred and whether deferoxamine therapy would extend the lifespan of patients. Further studies ultimately led to the licensing, by the Food and Drug Administration, of deferoxamine and of a portable infusion pump used to administer the drug, both of which were tested under NHLBI's programs. The noninvasive SQUID technique was developed for magnetically measuring the extent of iron accumulation in the liver. This instrument has been helpful in monitoring iron status and in identifying those patients who require more aggressive therapy because they are at risk of serious organ damage. Programs were begun for performing similar measurements on the heart.

In a similar fashion, innovative approaches to transfusion therapy have been established. Hypertransfusion therapy was shown to halt the ineffective production of the patient's red blood cells, which ameliorated the occurrence of facial and other bone deformities that resulted from the expansion of bone marrow cavities. In addition, hypertransfusion was shown to decrease the requirement for transfused blood by shrinking the total blood volume within the patient's body. This diminished requirement for transfused blood also decreased the rate of toxic iron accumulation. Methods were developed to separate the youngest fraction of a donor's red cells, and these "neocytes" were shown to last longer than whole blood when transfused into patients, thus resulting in a decreased requirement for transfused blood and a decrease in iron buildup. Serious inquiries into the cardiac evaluation of Cooley's anemia patients were begun, and important information regarding endocrine and organ function was obtained.

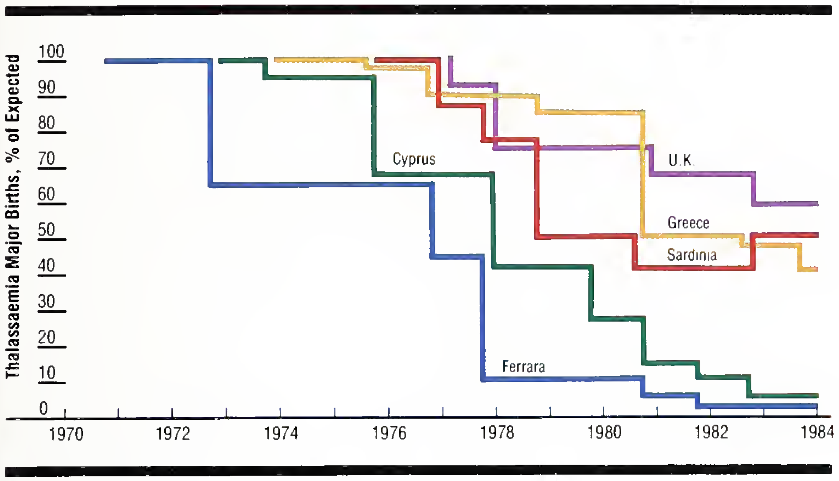
A minipump delivering the drug, deferoxamine, to a Cooley's anemia patient.

It now appears likely that deferoxamine treatment will prevent or delay the onset of cardiac disease caused by iron overload. This outcome appears to be especially apparent in patients who use the drug regularly. Recent unpublished data suggest that optimally treated patients survive significantly longer than before the current transfusion and chelation programs began.

Bone marrow transplantation holds promise for curing several disorders, including Cooley's anemia. Although bone marrow transplantation has been attempted in this country on a very limited scale with mixed success, in Italy, England, and Israel, the procedure has been used more extensively—about 150 transplants had been performed by the end of 1986. The major problems that prevent more widespread use of bone marrow transplantation are graft rejection, the likelihood of developing graft-vs-host disease (where the graft rejects the host), and the unavailability of suitable donors. Research in bone marrow transplantation using matched family members, nonidentical family members, and unrelated donors continues to be supported. Various approaches for controlling or eliminating graft-vs-host disease using immunosuppressive drugs and monoclonal antibody depletion of donor T cells, the cells of the immune system responsible for rejection reactions, continue to be investigated.

Education and Prevention Efforts

The NHLBI has been keenly aware of the importance of education and prevention programs for Cooley's anemia. In 1980, with the help and cooperation of the Cooley's Anemia Foundation, the NHLBI produced two prevention brochures, "Cooley's Anemia: Prevention Through Understanding" and "Cooley's Anemia: Prevention Through Testing." Hundreds of thousands of these brochures were distributed to schools, church groups, and civic and ethnic organizations. The interest that was generated sparked the beginning of local screening programs and created an awareness of Cooley's anemia and its prevention in the Italian and Greek communities.



Data from the World Health Organization illustrate the fall in the thalassemia major birth rate in five programs.

It is becoming clear that these efforts are beginning to yield encouraging results. The World Health Organization has reported marked decreases in the incidence of Cooley's anemia in several countries, including England, Greece, Italy, and Cyprus. Similar trends are being noted in the United States and Canada. Data from the 12 medical centers that provide care for the majority of Cooley's anemia patients indicate that 22 percent of the patients were below the age of 5 years in 1973, compared with only 11 percent in 1985. This striking decrease in the number of young patients is attributed to a decrease in the incidence of new cases. According to census data for Connecticut, this decrease has occurred in spite of a sharp increase in the Greek and Italian populations and no significant changes in the number of marriages involving at-risk couples. It reasonably can be concluded that changes in the reproductive behavior of at-risk couples account for this decrease in new cases.

The NHLBI is proud of the great strides that have been made in improving the quality of life of patients with Cooley's anemia. These advances are attributable, in part, to the special relationship and cooperation shared by the NHLBI, the National Cooley's Anemia Foundation, and the American-Hellenic Progressive Educational Association's Cooley's Anemia Foundation. Additionally, NHLBI has made a special effort to obtain advice from the scientific community. An expert panel was convened in 1978 and provided a report entitled "Assessment of Cooley's Anemia Research and Treatment." This publication identified important needs in patient care and research and served to guide the NHLBI in setting priorities and implementing programs. These recommendations were updated by a similar panel in 1982.

A major effort is underway to produce a comprehensive report of basic research, the clinical course of Cooley's anemia, therapy and management, epidemiology, psychosocial aspects, resource needs, and recommendations for future directions in research to guide NHLBI into the 1990s.

Amoz I. Chernoff, M.D.

Transfusion Medicine: Meeting The Challenge

Surgical procedures account for approximately 60 percent of the blood used in this country annually.

The National Heart Institute established the National Blood Resource Program in 1967. The program was based on the principle that donor blood should be treated as a national resource. There were many discernible problems in blood services that appeared to be resolvable through basic and applied research. No agencies in the private sector, however, were in a position, either financially or organizationally, to carry out the multifaceted national research program that would be required.

Some of the perceptions that motivated the founding of the National Blood Resource Program were:

- Blood transfusion is a therapeutic measure, sometimes lifesaving, for which there is no substitute in patients with acute blood loss.
- Blood transfusion always exposes the recipient to certain risks.
- Disease transmission, particularly hepatitis, was a frequent sequel to blood transfusion.
- Blood transfusion was administered far too often with little or no valid therapeutic need, thus exposing the recipient to wholly unnecessary risks.
- Whole blood was commonly used in clinical situations where only a particular component was indicated, thus not only exposing the recipient to unnecessary risks but also wasting components needed by other patients.
- The blood banking system was not producing components vitally important in the treatment of patients suffering from hemophilia, leukemia, and certain other diseases.
- There was no blood data system with which to assess the supply of and demand for blood and its components.
- There were intermittent shortages of donor blood and wastage of as much as 25 percent through outdating and maldistribution.
- There were no coordinated efforts among various voluntary, industrial, and governmental agencies involved in managing the blood supply.

Coordinating Research Efforts

Considerations such as these, as well as many others, provided the basis for the National Heart Institute's decision to establish a centrally coordinated program of research related to blood resources. The Institute used the contract mechanism in acknowledgment of the need for a goal-oriented approach to encourage and mobilize the research resources that existed and those that could be developed in universities, voluntary agencies, industry, and government.

While the problems were many and varied, there was reason for optimism that they could be resolved through coordinated research efforts. Some problems would require the stimulation of basic science studies for a solution, some data gathering, some applied developmental work, and others institutional arrangements. For instance, it was believed that the problems of wastage in the face of local and national blood shortages could be alleviated by systematization, regionalization, and cooperation among blood handling agencies, although such efforts would require a thorough system analysis. The most serious risk of transfusion was transmission of viral hepatitis, but prevailing research approaches held little promise that hepatitis carriers could be identified and eliminated from the donor pool. Additional encouragement and support were needed in the area of virological research to address this serious problem.

Furthermore, blood bank specialists had recognized for some time that there were not enough professionals to manage the increasingly complex needs of transfusion medicine. Thus an ongoing need was realized to train more scientists, managers, and allied health professionals in the intricacies of product development and appropriate blood use.

Blood component therapy, the practice of using only that specific cellular or plasma component needed by the recipient, was not yet widely practiced. Furthermore, the pharmaceutical industry was producing serum albumin from donor blood with the red cells, white cells, platelets, and plasma components, such as antihemophilic factor, almost totally wasted in the manufacturing process. The therapeutic value of platelet transfusion in leukemia and certain other forms of cancer was only beginning to be recognized and the means of producing and preserving platelet concentrates had not been perfected. Although it was known that the severe hemorrhagic crises suffered by many hemophilia patients were caused by insufficient quantities of a specific coagulation factor in their blood, the methods for isolating it from normal donor blood for subsequent supplementation therapy were not available. The techniques of manufacturing had to be developed, the effectiveness and safety of the product demonstrated, the quantitative need assessed, and the blood handling agencies and practicing physicians educated in its use.

Changing Concepts in Transfusion Medicine

In the 20 years since the inception of the National Blood Resource Program, many changes have occurred—organizational changes, scientific progress, and a new mode of operation for the entire system of transfusion medicine. The National Blood Resource Program grew rapidly and became the Division of Blood Diseases and Resources in 1972, one of the four current programmatic Divisions of the National Heart, Lung, and Blood Institute (NHLBI). Other important organizational changes also occurred throughout the country. The American Red Cross, traditionally a service organization, established a research laboratory with major capabilities in both basic

About eight million donors will donate approximately thirteen million units of blood in the U.S. in 1987.

and applied research relevant to transfusion medicine. The three national blood service organizations, the American Red Cross, the American Association of Blood Banks, and the Council of Community Blood Centers, became more and more influential in developing policies, consensus, and educational mechanisms for informing and instructing both producers (blood bankers) and consumers (practicing physicians) about rapidly evolving technologies and appropriate uses of blood components and blood products. In large measure because of efforts by the NHLBI, new research facilities were established, and new scientific personnel entered the field. Programmatic thrusts were initiated that led to Specialized Centers of Research in transfusion medicine, National Research and Demonstration Centers, and a unique training program referred to as the Transfusion Medicine Academic Award. The idea became accepted that donor blood is a national resource and, if managed properly, could improve the economy, safety, and efficacy of the blood supply and extend its benefits to a larger number and variety of patients than previously conceived.

In 1973, the Department of Health, Education, and Welfare promulgated the National Blood Policy embodying general principles upon which a national program should be built, and the American Blood Commission was established to oversee implementation of the policy. The NHLBI was designated as the lead agency to encourage wide acceptance of the National Blood Policy by supporting and funding specific efforts of the Commission. This body is composed of representatives of the major blood handling agencies and of several interested consumer groups. It carries on a continuing surveillance of blood handling systems, conducts seminars, and makes recommendations for modifications. The Commission provides a forum wherein consensus and cooperation can be achieved.

In any scientific endeavor, progress emanates from the efforts of many individuals. Each contribution becomes a building block in the total edifice; each is dependent on the work of others; and each provides a new level upon which others may build.

Since 1967, there has been spectacular success in improving the safety, efficiency, and availability of blood, blood components, and blood products. Major changes that have occurred over the past 20 years include serendipitous advances and spin-offs as well as progress resulting from carefully planned programs. For example, basic studies of erythropoietin, a substance in blood that stimulates the production of red cells in bone marrow, have led to its clinical application in the treatment of the anemia of chronic kidney disease. It now appears that one of the major demands for donor blood may be reduced drastically and that these patients no longer need be exposed to the multiple risks of hepatitis and other diseases transmitted by blood transfusion.

Considering the risks involved, it would appear that any efforts to reduce the transfusion of blood and blood components would be beneficial. Yet, the benefits of transfusion in terms of saving lives and restoring health are very great. Although significant effort is underway to develop and test artificial blood substitutes, such products are still in the experimental stages of development and not yet ready for clinical use in humans. The best policy at present is to use transfusion only when it is specifically indicated and never as a general supportive measure; to use only that fraction or component for which the patient needs replacement; to strive by all available means to identify and reduce the various elements of risk; and to build a service system or systems that will provide adequate amounts of high quality blood and blood components at reasonable cost and where needed.

Bar coding and computerization facilitate distribution of blood and blood components and reduce the potential for error.

Blood Resource Management

Great strides have been made toward fulfilling all of these goals. One of the important activities of the American Blood Commission was the establishment of the National Blood Data center, which provides information on trends in the blood collection and distribution system and information necessary for evaluating progress, assessing needs, and planning new emphases. Another important activity has been the development of guidelines for regionalization, which is defined as the organization of blood service units into geographically integrated areas to improve resource management, donor recruitment, and inventory control.

The increased sophistication of inventory control in regional centers, made possible by computerization, has resulted in a substantial reduction of wastage through outdating in this country. In regional centers, blood can be distributed and redistributed among user hospitals providing the advantages of both immediate, onsite availability and timely identification of additional supplies of specific types within the region. In effect, a unit of blood is given maximal exposure to potential users during its shelf life. Reduction in wastage obviously means that the need for unit donations will be reduced and that the limited blood supply will be used more effectively.

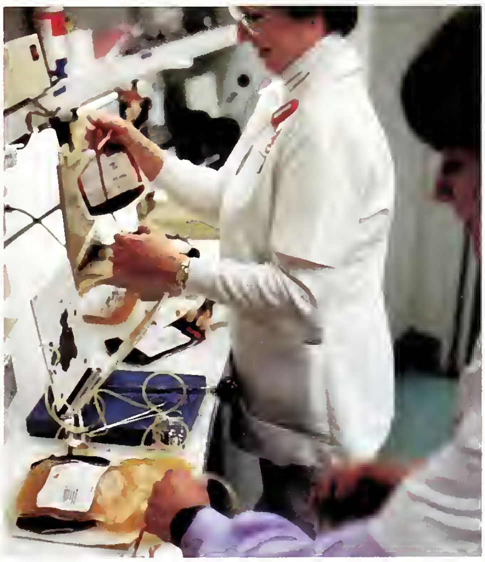
Another development of perhaps even greater impact has been the extension of the shelf life of blood from the traditional 21 days to 35 days as a result of improvements in preservative solutions. Again, improvements such as these increase the probability that a given unit of blood will be utilized.

Blood bank nurse supervisor processing red blood cells for transfusion.

Blood Component Therapy

A radical change in the economy of blood used during the past 20 years has been the introduction and expanded use of blood components rather than whole blood. Prior to 1967, the great majority of donor blood was used for whole blood transfusions in a variety of surgical and medical situations in which restoration of the recipient's red cell mass was desired. Now, it is widely recognized that this objective is served by giving the patient only the red cells that have been separated from plasma. The prevailing opinion is that there are few clinical indications in which the use of whole blood is preferable to red cells alone. Exceptions, of course, would be cases in which the need is so emergent that there is no time for the separation of blood into components or in which immediate, large-volume replacement is indicated for patients who are bleeding extensively. Although whole blood still is transfused in some places, the practice has declined significantly in this country.

Technicians separating individual blood components.



The separation of red cells from plasma and cellular elements provides a large resource from which to obtain other therapeutically useful elements. Prior to 1967, the only product utilized on a significant scale was albumin, which was prepared by the pharmaceutical industry from either outdated bank blood or blood purchased specifically for albumin production. With the discovery that a potent antihemophilic factor concentrate could be prepared from fresh-frozen plasma, new impetus was given to the practice of plasma fractionation, which offered the advantage that an individual unit of blood could be used to

Over four million platelet concentrates will be transfused in 1987.

provide red cells for one patient and the missing clotting factor for the treatment of another patient. Almost simultaneously, methods were developed in university and pharmaceutical company laboratories to produce antihemophilic factor from plasma pooled from many donors, thus yielding large quantities of concentrate of standardized potency.

Other applications of component therapy followed. It was demonstrated that the often fatal hemorrhaging of leukemia patients could be controlled by the transfusion of blood platelets concentrated from donor blood. At first, it was necessary to use the platelets within 24 hours after blood donation because these fragile cells deteriorated rapidly during storage. However, improvements in methodology have made it possible to store platelets for as long as 5 days. It was observed, however, that platelet transfusions were not always effective and that the platelet destruction observed in the recipient's circulation was caused by platelet alloimmunization. As a result of these observations, donor-recipient matching was introduced, and although large numbers of matched donors are not easily found, an individual donor can provide more than adequate amounts of platelets for a matched recipient. Apheresis techniques have been developed that provide the means of obtaining sufficient quantities of platelets from individual donors. In this procedure, the donor's blood passes through a closed, extracorporeal circuit and is centrifuged; selected cells are harvested, and the remaining elements are returned to the donor. This new technology ushered in a new era of component therapy. Concentrates of specific cells or plasma now are easily obtained and available for specific clinical needs with tremendous cost savings.

Blood Substitutes

During the past decade, research on artificial blood substitutes has focused primarily on oxygen-carrying red cell substitutes, namely, perfluorochemical emulsions and hemoglobin solutions. Blood substitutes offer many advantages over natural blood

Donor undergoing continuous-flow plateletapheresis.

products. Patients who require rare blood types that are not readily available would be able to rely upon artificial preparations. Similarly, artificial preparations would be extremely useful in times of disaster because they would not require typing, could be stored in large quantities for extended periods of time, and would be free of infectious agents. Also, for therapeutic procedures in which large volumes of blood are used, an artificial substitute would conserve natural blood and its components for other critical needs. While the NHLBI has supported the synthesis and screening of new perfluorochemical compounds and the development of knowledge concerning the physiological consequences of partial and total blood replacement, a clinically useful perfluorochemical preparation continues to be a goal for the future. Studies are in progress on the use of stroma-free hemoglobin solutions as blood substitutes, including an evaluation of "neohematocytes" that are composed of hemoglobin molecules encapsulated in lipid membranes. Scientists have investigated the use of perfluorochemicals for therapeutic interventions other than acute blood loss. Such efforts include the use of perfluorochemical preparations in organ perfusion and preservation, magnetic resonance imaging, and a variety of basic research investigations. Also, results from human clinical trials, in which these compounds have been used to sensitize solid tumors prior to irradiation and as an adjunct to coronary angioplasty, have been extremely encouraging. Artificial blood substitutes have thus progressed from what was only an idea over a decade ago to a potential clinical reality. Other strategies for the more economical use of the national blood resource have involved studies on how to improve blood bank management, donor recruitment, and donor motivation.

Technician determining the HLA type of a bone marrow donor.

Automated blood typing reduces the possibility of human error.

Safety of Transfusion Therapy

Thus far, we have been concerned with the economy and adequacy of the blood resource. Another great concern is safety. In transfusion medicine, there are many possible sources of risk to the blood recipient, including bacterial contamination of blood during processing, intoxication of blood with materials used during processing, mismatching of blood, clerical error, and physical damage to blood during storage. However, the most important and difficult problem is the transmission of infectious diseases from donor to recipient. The principal diseases known to be transmitted by blood transfusion are syphilis, malaria, and viral diseases, such as the various forms of hepatitis, acquired immunodeficiency syndrome (AIDS), and cytomegalovirus infection. The first two disorders are controlled to a satisfactory degree by the careful screening of donors by medical history and serologic testing. The viral diseases, however, have proved to be much more difficult to detect because the potential donor is often a normal or "silent" carrier of the virus. During the late 1960s, virus researchers identified hepatitis B, the agent responsible for serum hepatitis. In the years that followed this discovery, a number of strategies were employed to deal with the problem. Researchers worked to develop tests to detect hepatitis B viral antigen in blood, produce hyperimmune globulin for therapeutic trials, develop methods to remove virus from blood, assess the value of hyperimmune globulin in the prevention of hepatitis in those accidentally exposed, and study the natural history of the disease in prospective clinical trials. The prevalence and variety of epidemiologic characteristics of the disease were displayed dramatically in a controlled study in which heart surgery patients, who required multiple units of blood, were randomized to receive either volunteer blood from NIH employees or blood purchased from a commercial bank operating in a low-income area. More than 50 percent of the recipients who received commercial blood developed evidence of virus transmission, whereas none of the recipients of volunteer blood became infected.

By the early 1970s, reliable serologic tests for mass screening of donors were licensed and introduced into all blood banks. It appeared that the problem of hepatitis transmission by blood transfusion would be resolved by eliminating donors who tested positive for the presence of the virus.

In the late 1970s, a vaccine was prepared and shown to be effective in the prevention of type B hepatitis, a significant achievement of great public health importance. Unfortunately, however, it became apparent that only about 30 percent of posttransfusion hepatitis was attributable to type B hepatitis virus. A new disease category termed non-A, non-B hepatitis was recognized that is caused by one or more as yet unidentified viruses. Between 1974 and 1979, the NHLBI sponsored the Transfusion-Transmitted Virus (TTV) study, which was a multihospital cooperative study of posttransfusion hepatitis. This project focused on the surveillance and etiology of transfusion-associated viral hepatitis and provided evidence for the existence of non-A, non-B hepatitis virus(es). The TTV study and several other regional studies have revealed that approximately 7 to 10 percent of all blood recipients develop non-A, non-B hepatitis and that the transfusion of blood and blood products to over 3 million recipients annually results in approximately 300,000 cases of non-A, non-B hepatitis in the United States each year. Another important contribution of the TTV study was the discovery of an association between donor serum alanine aminotransferase (ALT) levels and recipient non-A, non-B hepatitis. Similarly, an association between antibody to hepatitis B core antigen in donor blood and recipient non-A, non-B hepatitis was also established. These findings have had important implications for blood transfusion services, and the major blood collecting organizations have adopted the policy of screening all units of donated blood for ALT and antibody to hepatitis B core antigen. The TTV study also showed that chimpanzees were susceptible to non-A, non-B infection, thus identifying an animal model that has become invaluable in the study of the pathogenesis of non-A, non-B hepatitis. Results from studies to develop and evaluate tests to detect non-A, non-B hepatitis virus(es), however, have been disappointing because all attempts to isolate and characterize the agent(s), a prerequisite for the development of an assay, have failed. Nevertheless, it is estimated that approximately 50 percent of posttransfusion non-A, non-B hepatitis may be prevented by screening donated blood for ALT and antibody to hepatitis B core antigen.

Laboratory technician screening donated blood for human immunodeficiency virus antibodies.

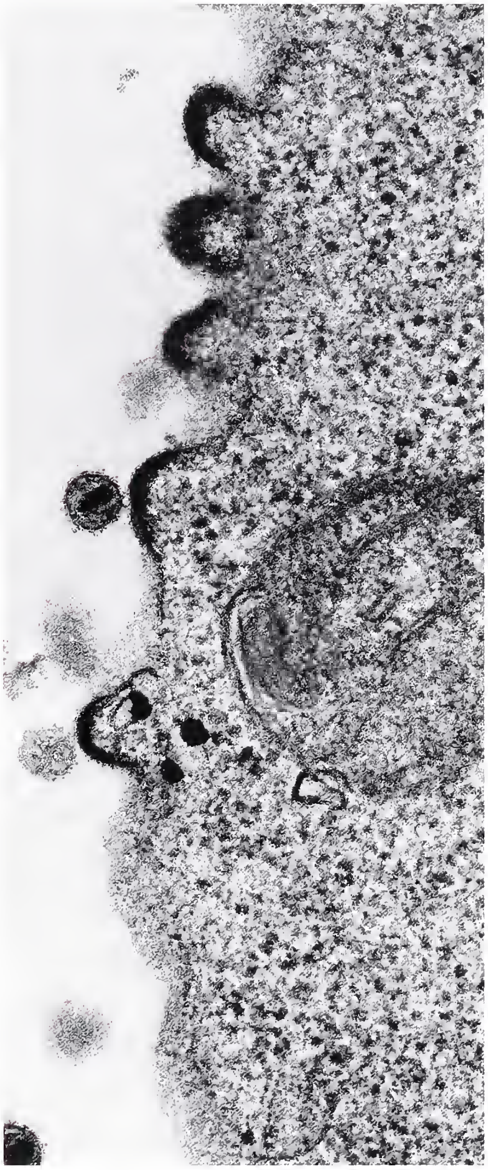
The hepatitis transmission problem has been particularly agonizing in the hemophilia population. A typical lot of antihemophilic factor concentrate may be derived from the blood of thousands of donors. In such cases, the chance that one or more of the donors may be a carrier of the virus is great. In fact, the vast majority of severe hemophiliacs who receive frequent treatment with factor concentrates have become infected with various hepatitis viruses. More recently, research has shown that heat or chemical treatment of the preparation will inactivate some of these viruses. Consequently, heat or chemical inactivation procedures have been introduced into standard manufacturing practice.

Great efforts have been made to prepare effective vaccines to protect high-risk individuals from hepatitis B infection. The first approach was to prepare a vaccine from the plasma of hepatitis B virus carriers. More recently, a genetically engineered hepatitis vaccine has been developed and approved for use in humans.

In the early 1980s, a new and more serious problem confronted the transfusion medicine community. AIDS appeared in the American population, and it soon was shown to be caused by a virus termed human immunodeficiency virus (HIV) that could be transmitted by blood and blood products.

An alarming characteristic of HIV infection is that there are many carriers of the virus who do not manifest symptoms of the disease but are highly capable of transmitting the agent. Initially, the blood banks' only recourse was to ask donors to disqualify themselves if they exhibited certain high-risk behaviors, a slender reed indeed upon which to rely for defense against this dread disease. In the spring of 1985, a screening test for the antibody to HIV was introduced into all blood banks across the country. The test has a high degree of accuracy, and a positive result corroborated by a second confirmatory test indicates that an individual has been infected with the virus and is probably still carrying the agent. These screening procedures have been extremely effective and have almost completely eliminated the transmission of HIV by blood and blood products.

Initial reports of the possible transmission of AIDS via transfusion, particularly in the hemophilia population, led to important studies of an epidemiologic nature, such as the Transfusion Safety Study. Efforts to develop direct and indirect tests to identify carriers were accelerated, as was research to achieve a means for inactivating the virus responsible for the disease. Chimpanzee research led to the development of the first animal model for HIV infection. Currently, there is much effort to educate both the donor population and the professional user of blood and blood products in all aspects of the proper collection and use of the blood resource.



Human immunodeficiency virus particles budding off the cellular membrane of a lymphocyte.

The problem of AIDS remains very serious. In the world of transfusion medicine, the catastrophic effect of the AIDS problem first was observed in the hemophilia population. HIV transmission in the hemophiliac is in many ways analogous to hepatitis transmission in that group. In both cases, commercially available antihemophilic concentrates upon which many hemophiliacs are dependent for their lives and well-being, as stated previously, are prepared from the pooled plasma of a large number of donors. This means that patients who receive factor concentrates are exposed to a very large number of donors, some of whom may be infected carriers. Much of this exposure occurred before the medical community was even aware of AIDS and much of it before the protection afforded by the screening test to detect the antibody to HIV was in place. In fact, it is likely that the great majority of hemophiliacs have been exposed to the virus. By February 1987, 254 cases of AIDS in hemophiliacs had been reported, and of those, 147 had died. In addition to these tragic morbidity and mortality figures, fear of the disease has permeated almost all facets of society.

There is evidence, as in the case of hepatitis virus(es), that heat or chemical treatment of blood products is effective in inactivating HIV. The fact that such procedures are effective and that screening measures are in place to eliminate carriers from the donor population provides the needed assurance that antihemophilic preparations now are probably free of infectious virus. Research aimed at developing chemical treatment of blood products to inactivate viruses of both hepatitis and AIDS has

Testing for antibody to cytomegalovirus.

shown great promise. Studies are in progress to develop an AIDS immune globulin derived from the blood of individuals carrying the antibody. Preliminary indications are that it might be useful in preventing the disease in high-risk individuals. The greatest hope, of course, is that it will be possible to develop an effective vaccine. Many researchers are engaged in such efforts using a variety of approaches.

Another virus that is transmissible by blood transfusion is cytomegalovirus, which is almost ubiquitous in the adult population but produces little clinical disease. Premature infants who receive blood transfusions, however, suffer about a 25 percent risk of cytomegalovirus infection and overt disease, which may be fatal in approximately 20 percent of these recipients. There is, at present, no effective means to either prevent or treat this disease. Presumptive evidence is that neonates are at high risk because they have not yet produced antibodies and may not have maternally-produced antibodies. A randomized trial of the efficacy of passive immunization is now underway. Cytomegalovirus immune globulin was prepared from normal donor plasma, which contains high titers of antibody to cytomegalovirus. This antibody is given intravenously to test subjects at the time of blood transfusion and at intervals thereafter. The trial will determine whether passive immunization can protect the premature neonate against this transfusion-related disease.

Two projects were conceived in the mid-1970s to provide the scientific community with unique resources for research on transfusion-associated infections. Since 1974, the NHLBI has supported a sizable colony of chimpanzees for studies judged most likely to advance hepatitis research and, more recently, AIDS research. As the only animal to which non-A, non-B hepatitis or HIV can be transmitted, even experimentally, chimpanzees constitute an invaluable resource to investigators concerned with these transfusion-transmitted diseases. Another important resource for researchers is a blood specimen repository containing over 300,000 serum and cellular specimens. The repository maintains specimens collected in multi-institutional prospective studies of the epidemiology of posttransfusion hepatitis and AIDS. Since the establishment of this resource in 1975, aliquots of samples have been used by numerous scientists in the performance of studies concerned with transfusion-transmitted hepatitis, AIDS, cytomegalovirus, and other infectious agents.

In 1985, the first Specialized Centers of Research and National Research and

The NHLBI maintains a chimpanzee colony for nondestructive studies of posttransfusion hepatitis or AIDS.

Demonstration Centers in Transfusion Medicine were established. These "centers of excellence" were established to expedite the development and application of new knowledge with the goal of improving the availability, safety, and quality of blood and blood products for therapeutic uses. They conduct a full range of research and development activities from basic to clinical. They also provide the ideal environment for basic scientists, blood bank scientists, engineers, and clinicians to interact, exchange ideas, and become aware of the problems within the field of transfusion medicine to expedite research progress in this critical area.

For over a decade, special attention has been focused on training scientists for research in transfusion medicine because the field has expanded and increased in complexity. The number of blood preparations routinely used has increased from three or four to more than two dozen within two decades and, as already stated, the uses of transfusion have evolved from the simple transfusion of whole blood for emergency purposes to infusion of multiple blood components and derivatives for a large number of acute and chronic diseases. These advances, plus the continuing presence of the hepatitis threat and the emergence of AIDS, have served to highlight the need for more trained scientists and physicians who can address present problems and future needs.

Training Programs in Transfusion Medicine

To expand the pool of scientists, special programs, such as the Special Emphasis Research Area (SERA) in the Blood Banking Sciences and the Transfusion Medicine Academic Award (TMAA), have been developed. SERA encouraged scientists from related disciplines to receive special training that could be applied to blood resources problems. The TMAA was initiated in 1983 to encourage the development of education programs in transfusion medicine. The purpose of the TMAA is to encourage schools of medicine, in cooperation with local blood centers, to organize curricula in the discipline of transfusion medicine and to further the development of skilled personnel who can serve the research, teaching, and clinical needs of transfusion medicine.

The foregoing discussion provides only brief highlights of a 20 year span. During that time, a large number of studies have been undertaken to improve the safety, efficacy, and availability of the Nation's blood resource. The studies are too numerous and too complex to detail in a short report. They have incorporated the entire array of scientific endeavor from the most basic to the most applied; from molecular studies to social research; communication, conferences, and consensus sessions; the establishment and support of laboratories and research centers; the building of serum and data banks important to epidemiologic studies; the creation and support of a chimpanzee colony vital to studies of the pathogenesis of viral diseases transmitted by blood; the training of scientific personnel; and the introduction of transfusion medicine curricula into schools of medicine. This research has brought great change in the medical community and provided incalculable health benefits to the American people.

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